Systolic Ejection Murmurs and the Left Ventricular Outflow Tract in Boxer Dogs

Physiology and Clinical Evaluation

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Abstract

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Turbulence of various genesis in the left ventricular outflow tract (LVOT) causes systolic ejection murmurs. The prevalence of murmurs in adult boxer dogs is 50-80%, the majority of which are of low intensity. Some of the murmurs are caused by aortic stenosis (AS), while the origin of the others is unclear. The aim of this thesis was to study the physiology and clinical evaluation of systolic ejection murmurs and their relation to the development of the LVOT in boxers with and without AS.
Growing and adult boxer dogs were examined by the standard methods cardiac auscultation, ECG, phonocardiography and echocardiography. Additionally, the complementary methods time-frequency and complexity analyses of heart murmurs and contrast echocardiography were evaluated.
Studies on inter-observer variation in cardiac auscultation proved the importance of experience in detection and grading of low intensity ejection murmurs. Excitement of the dogs by exercise or noise stimulation (barking dog and squeaky toy) caused higher murmur grades, longer murmur duration and increased aortic flow velocities. No differences were found between diameters measured at different levels of the LVOT in growing boxers. Contrast echocardiography enhanced Doppler signals, but did not allow evaluation of myocardial blood flow. Using time-frequency analysis, duration of murmur frequency >200 Hz proved useful for differentiation between dogs with mild AS and dogs without. Combining assessment of murmur duration >200 Hz and complexity analysis using the correlation dimension (T_2), a sensitivity of 94% and a specificity of 82% for differentiation between dogs with and without AS was achieved. The variability in presence and intensity of low intensity murmurs during growth was high. None of the young dogs developed AS, whereas 3 out of 16 individuals developed mild-moderate aortic insufficiency. Aortic or pulmonic flow velocities did not differ significantly between growing dogs with or without low intensity murmurs.
In conclusion, the variability in presence and intensity of low intensity ejection murmurs in boxers is high during growth with no obvious progression. Both in young and adult boxers the murmur grade increased during excitement, which may be due to rapid flow in a comparatively small LVOT that has been suggested for the boxer breed. Experience is important in cardiac auscultation of low intensity murmurs. Therefore, assessment of murmur duration > 200 Hz combined with T_2 analysis may be a useful complementary method for diagnosis of cardiovascular function in dogs.

Key words: systolic ejection murmur, aortic stenosis (AS), aortic insufficiency (AI), left ventricular outflow tract (LVOT), boxer dog, auscultation, phonocardiography, echocardiography, murmur analysis

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To My Family

Contents

Appendix

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. K. Höglund, A. French, J. Dukes-McEwan, J. Häggström, P. Smith, B. Corcoran, C. Kvart. Low intensity heart murmurs in boxer dogs: Inter-observer variation and effects of stress testing. *Journal of Small Animal Practice* 2004; 45: 178-85

II. K. Höglund, C. Bussadori, O. Domenech, J. Häggström, D. Pradelli, C. Kvart. Contrast echocardiography in Boxer dogs with and without aortic stenosis. *Journal of Veterinary Cardiology* (In press)

III. K. Höglund, C. Ahlstrom, J. Häggström, P. Ask, P. Hult, C. Kvart. Time-frequency and complexity analyses for differentiation of physiologic murmurs from heart murmurs caused by aortic stenosis in Boxers. *American Journal of Veterinary Research* (In press)

IV. K. Höglund, J. Häggström, C. Bussadori, C. Kvart. A prospective cohort study of systolic ejection murmurs and the left ventricular outflow tract during the growth period in boxer dogs. (Submitted)

Abbreviations

The following abbreviations are used in the text:

2D	Two-dimensional
AI	Aortic insufficiency
AS	Aortic stenosis
AV	Atrioventricular
ECG	Electrocardiography
EOD	Effective orifice diameter
HR	Heart rate
κ	Kappa
LVOT	Left ventricular outflow tract
PCG	Phonocardiography
SA	Sinoatrial
SAS	Subvalvular aortic stenosis
SD	Standard deviation
TFP	Time-frequency properties
T_2	Correlation dimension
VFD	Variance fractal dimension

Introduction

Physiology of the heart

In 1628, William Harvey claimed that 'blood by the beats of the ventricles flows through the lungs and heart and is pumped to the whole body'. Based on his research, Harvey thereby overthrew the predominant view of the heart as the source of body heat, proving instead that the heart is a muscular pump providing both lungs and the rest of the body with blood (Katz, 2006). The heart has four pumping chambers, which are separated by the septa and valves. The atria and ventricles are separated by the atrioventricular valves - the mitral valve on the left side and the tricuspid valve on the right. The great arteries are separated from the ventricles by the aortic valve on the left side and the pulmonic valve on the right. These valves, assisted by the venous valves, keep blood flowing in one direction in the circulatory system. All four cardiac valves lie in a plane within connective tissue that separates and insulates the atria from the ventricles. The semilunar aortic and pulmonary valve cusps are supported by thick tendinous margins. Behind each of the three aortic valve cusps lies the Sinus of Valsalva, containing the orifices of the coronary arteries, which supply the myocardium with oxygenated blood (Katz, 2006). The aorta has a large diameter and thick walls, which contain a large proportion of elastic tissue, allowing the aorta to expand in response to increased pressure (Junqueira & Carneiro, 2005; Guyton & Hall, 2006).

The cardiac muscle is composed of myocytes and a connective tissue matrix. Each myocyte consists of numerous myofibrils comprising longitudinally arranged sarcomeres. The sarcomeres are the functional contractile units of the striated heart muscle, consisting of thick myosin filaments and thin filaments containing actin, tropomyosin and troponin. Contraction of the sarcomere is effected by sliding of the filaments along one another, a process dependent on energy and calcium concentration within the myofibril (Kittleson & Kienle, 1998).

Activation of cardiac contraction begins in the sinoatrial (SA) node (Anderson & Ho, 1998), spreading through the atrial myocardium before the depolarization wave is delayed in the atrioventricular (AV) node. Next the ventricles depolarize via the AV-bundle, the left and right bundle branches and the His-Purkinje-system, which is a subendocardial network of rapidly conducting cells synchronizing ventricular activation (Guyton & Hall, 2006; Katz, 2006).

In response to the electrical depolarization of cells, the heart contracts and relaxes in a cyclical fashion, called the cardiac cycle. During the period of ventricular relaxation (diastole), the semilunar valves are closed; the atrioventricular valves open and blood flows from the atria into the ventricles. During ventricular contraction (systole), the pressure rises within the ventricles, causing the atrioventricular valves to close. When pressure inside the ventricles exceeds that of the great arteries, the semilunar valves open and ventricular ejection starts. Ejection lasts until the end of systole, when the semilunar valves close and a new cycle starts (Kittleson & Kienle, 1998). During systole the pressure in the aorta increases, which leads to expansion of the elastic vessel and increased blood flow (Guyton & Hall, 2006).

The heart is richly innervated, both by the sympathetic and parasympathetic nervous systems (Katz, 2006). The heart rate (HR) is determined by the rate of SA nodal depolarisations. Parasympathetic stimulation through the vagal nerve slows the SA nodal discharge as well as the AV nodal conduction and decreases cardiac output, while the sympathetic nervous system has the opposite effect. In a normal dog, the HR is approximately 100 beats/min, but this can vary between 45 beats/min at rest and around 260 beats/min during peak exercise (Hamlin, 1999).

Heart sounds and murmurs

Heart sounds and murmurs are low intensity sounds of which only a small segment is audible to the human ear. The transient heart sounds generated during the cardiac cycle originate from vibrations of cardiac structures, initiated by rapid acceleration or deceleration of blood (Sisson & Ettinger, 1999; Katz, 2006). The first heart sound, S1, occurs when vibrations are caused by closure of the atrioventricular valves and rapid increase in ventricular pressure as the ventricles start to contract. S1 signals the start of mechanical systole, which is terminated by closure of the semilunar valves, creating vibrations causing the second heart sound, S2 (Kvart & Häggström, 2002). The relationship between the S2 and the closure of the aortic and pulmonary valves was first demonstrated in the 1830s by James Hope, a British cardiologist performing open-chest experiments in a stunned donkey (Leatham, 1987; Hanna & Silverman, 2002). Characteristically, S1 is louder, longer and lower pitched, compared to S2 (Sisson & Ettinger, 1999). The third heart sound, S3, originates from early and rapid diastolic filling of ventricles resistant to expansion, and should not be present in healthy small animals since it is indicative of myocardial failure (Sisson & Ettinger, 1999; Kvart & Häggström, 2002; Prosek, 2005). Finally, the fourth heart sound, S4, is generated during atrial contraction and again is a sound that should not be present in healthy small or medium-sized dogs with normal-sized atria (Kvart & Häggström, 2002; Prosek, 2005).

Heart murmurs originate from turbulence in the blood flow, which causes vibrations in the heart and adjacent blood vessels, and are sounds of longer duration than the transient heart sounds (Prosek, 2005). The tendency for turbulent flow increases in direct proportion to the velocity of blood flow, the diameter of the blood vessel and the density of blood, and is inversely proportional to the viscosity of the blood according to the following equation:

$$\text{Reynold's number} = \frac{\text{Velocity} \times \text{Diameter} \times \text{Density}}{\text{Viscosity}}$$

Hence, Reynolds number is a measure of the tendency for turbulence to occur. At low Reynold's numbers flow is laminar, whereas turbulent flow occurs when the number reaches a critical high level, causing blood cells to travel at multiple velocities and in different directions (Kittleson, 1998; Prosek, 2005; Guyton & Hall, 2006).

Heart murmurs are described by a number of factors: timing in the cardiac cycle, intensity on a scale of I-VI, shape, frequency, point of maximal intensity and radiation (Gompf, 1988; Kittleson, 1998; Sisson & Ettinger, 1999; Kvart & Häggström, 2002). Classification of systolic heart murmurs are given in table 1.

Table 1. *Classification of systolic heart murmurs and examples of associated conditions.*

Classification	Intensity	Characteristic	Association
Innocent/ physiologic	I-II/VI	Early systolic ejection	Puppies Thin-chested dogs
Functional	I-III/VI	Early-mid systolic ejection	Anaemia Fever Hyperthyroidism High cardiac output
Pathologic obstruction	II-VI/VI	Crescendo/decrescendo, duration 50-100% of systole	Aortic or pulmonic stenosis
Pathologic regurgitation	I-VI/VI	Band-shaped holosystolic	Acquired valvular disease Dilated cardiomyopathy

Innocent or physiologic murmurs are most commonly detected in young animals without structural evidence of heart disease, and in most dog breeds these murmurs diminish or resolve by the age of 6 months (Kvart & Häggström, 2002; Oyama *et al.*, 2005; Prosek, 2005). One possible reason for this is that puppies tend to have larger stroke volumes for the size of their great arteries compared to adult dogs (Kittleson, 1998). Functional murmurs may be present in dogs with diseases in other organ systems, but without structural heart disease. Other physiological conditions which may induce functional murmurs include pregnancy and increased sympathetic tone, both by increasing the cardiac output (Sisson & Ettinger, 1999; Prosek, 2005).

Heart murmurs caused by cardiac disease are most commonly due to high-velocity flow through a narrow orifice. Valvular regurgitation and obstruction of blood flow by aortic or pulmonic stenosis are examples of conditions which can cause these murmurs (Kittleson, 1998; Kvart & Häggström, 2002). Pathological murmurs generally have a longer duration compared to nonpathological murmurs (Kvart & Häggström, 2002). However, milder pathological murmurs may be difficult to distinguish from innocent or functional murmurs (Sisson & Ettinger, 1999).

Systolic ejection murmurs are defined as murmurs of crescendo-decrescendo type, beginning with ejection shortly after the onset of systole. These murmurs can be caused by a pathologic obstruction or by other conditions causing increased flow or decreased blood viscosity (Kittleson, 1998).

Cardiovascular diagnostic techniques

The art of investigating sounds from the chest of patients has a long history. Hippocrates, the Greek physician often referred to as 'The Father of Medicine', was known to practice auscultation by placing his ear directly on the patient's chest (Abdulla, 2001; Hanna & Silverman, 2002). Needless to say, this method was not very efficient. The stethoscope (derived from the Greek words stéthos, meaning chest, and skopé, meaning examination) was invented in 1816 by a French physician named Rene Theophile Hyacinthe Laënnec. By investigating thoracic sounds and comparing them to observations made during autopsies, he developed the stethoscope into a useful diagnostic tool, an invention which has contributed greatly to advances in to the study and diagnosis of chest diseases (Roguin, 2006). Over the years since the introduction of Laënnec's monaural wooden tube, the stethoscope has been developed and refined by several inventors, leading to the modern binaural acoustic stethoscope designed by Rappaport, Sprague and Groom in the 1940s. Today's equipment is characterised by a combination chest piece, short rubber tubing and well fitting earpieces (Roguin, 2006). The use of the stethoscope for cardiac auscultation thereby provides an efficient way of listening to heart sounds and murmurs. However, the interpretation of the sounds is subjective and dependent on the expertise of the examiner (Tavel, 1996).

Phonocardiography (PCG) was initially developed by Einthoven in 1894 (Durand & Pibarot, 1995) whose aim was to provide the physician with a complementary tool to cardiac auscultation, by providing a permanent written graphic recording of heart sounds and murmurs. Combining the PCG with an electrocardiogram (ECG) makes it possible to appreciate the timing and relation of heart sounds and murmurs to each other (Hanna & Silverman, 2002). In the late 1940s, the British cardiologist Aubrey Leatham started using a PCG equipped with a low frequency filter in order to mimic the acoustic properties of the human ear and the stethoscope. This prerequisite for high quality PCGs was identified also by Rappaport and Sprague (Leatham, 1987), and as a result most PCG machines record sounds with frequencies between 50-500 Hz (Ettinger & Suter, 1970).

The invention of the electrocardiogram (ECG) was based on the discovery that the beating heart generates an electrical current (Kolliker & Müller, 1856). By 1900, Einthoven had developed the string galvanometer (Katz, 2006), thereby making the ECG a practical clinical tool. The ECG records the average electrical potential generated in the myocardium during the cardiac cycle, which is then graphically displayed in voltage and time (Miller *et al.*, 1999). It is a non-invasive, simple, reproducible and cheap means of assessing heart rhythm, heart rate and mean electrical axis (Fisch, 2000).

X-rays were discovered by Wilhelm Conrad Röntgen in 1895 (Frankel, 1996). Thoracic radiography provides information about cardiovascular size and shape, and can be used to observe abnormalities in lung parenchyma and airways. It can also be used for indirect assessment of the cardiopulmonary circulation (Lord & Suter, 1999).

Direct assessment of cardiac circulation was previously made by cardiac catheterisation, an invasive technique in which a catheter is inserted into a chamber or vessel of the heart in order to measure intracardiac and intravascular pressures, cardiac output and oxygen content of the blood (Ettinger & Suter, 1970). The first echocardiogram was registered by the Swedish cardiologist Inge Edler and his co-worker, physicist Hellmuth Hertz in 1953 (Edler & Hertz, 2004). Since then, there has been an enormous development within the field and echocardiography is now an integral part of clinical cardiology. This technique provides accurate, non-invasive assessment of cardiac structure, function and blood flow dynamics by use of ultrasound waves (Moise & Fox, 1999). Ultrasound is produced when piezoelectric crystals in a transducer are electrically stimulated and the ultrasound beam is transmitted into tissues as a propagated wave causing particles in the tissue to oscillate. When sound waves encounter acoustic interfaces, they are reflected back to the transducer producing electrical energy and an image is created on a monitor screen. In this way, 2-dimensional structures and motions can be studied. The Doppler echocardiographic technique uses scattering from moving blood cells for evaluation of direction and velocity of blood flow. Spectral Doppler examinations include pulsed-wave and continuous-wave Doppler for velocity measurements, while colour Doppler is used for detection of turbulence and valve leakage by colour-coding the direction of the blood cells (Boon, 1998; Moise & Fox, 1999). Tissue Doppler imaging (TDI) is a novel ultrasound modality used for assessment of regional myocardial motion (Chetboul *et al.*, 2006a). Today, cardiac ultrasound and Doppler echocardiography have made diagnostic cardiac catheterization almost unnecessary (Noonan, 2004).

In recent years, there have been an increasing number of investigations into the use of cardiac biomarkers for the diagnosis of cardiac disease and heart failure in veterinary medicine (Häggström *et al.*, 1997; Tidholm, Häggström & Hansson, 2001; Oyama & Sisson, 2004; Prosek *et al.*, 2004; Oyama, Sisson & Solter, 2007). These circulating substances tend to be either involved in the systemic neurohumoral response to heart failure (natriuretic peptides, angiotensin II and endothelin) or released from injured myocardial tissue (*e.g.* troponins), (Boswood, 2004). Some cardiac biomarkers are already used diagnostically as indicators of acute myocardial injury or presence of heart disease, such as cardiac troponin I and N-terminal fragment of pro-brain natriuretic peptide (NT-proBNP), while others are still under investigation (Oyama & Sisson, 2004; Oyama, Sisson & Solter, 2007).

Magnetic resonance imaging (MRI) uses non-ionizing radio frequency signals to demonstrate pathological or physiological alterations of living tissues. Due to the large field of view and its capability of direct imaging in multiple planes this non-invasive image technique can be useful for heart morphology evaluation (Higgins *et al.*, 1984; Winterer *et al.*, 1999).

Aortic stenosis

Aortic stenosis (AS) is a common congenital heart disease in dogs, most frequently seen in large-breed dogs (O'Grady *et al.*, 1989; Tidholm, 1997; Kienle, 1998). The most commonly reported form is subvalvular aortic stenosis (SAS). Valvular AS also occurs in dogs, either separately or in combination with the subvalvular form. Supravalvular AS is considered rare in dogs (Kienle, 1998; Bonagura & Lehmkuhl, 1999).

SAS is characterised by narrowing of the left ventricular outflow tract (LVOT), resulting from a fixed ridge or ring of fibrocartilaginous tissue located in the left ventricular outflow tract just below the aortic valves (Oyama, *et al.*, 2005). It has been suggested that the fibrocartilaginous ring of SAS is derived from persistent embryonal endocardial tissue which retains its proliferative capacity for some time after birth. In post-mortem studies of Newfoundlands affected by SAS, the lesions were graded from 1-3, ranging from small, slightly raised nodules on the endocardial surface to a fibrous band, ridge or collar completely encircling the LVOT (Pyle, Patterson & Chacko, 1976). Valvular AS is characterised by thickening of the aortic valve leaflets and obstruction of the blood flow by incomplete opening of the valves during systole (Bonow & Braunwald, 2005).

The aortic stenosis causes blood flow velocity to increase and distal to the obstruction the sudden increase in cross-sectional area results in turbulent blood flow (Nygaard *et al.*, 1993)**.** The turbulence creates a systolic ejection murmur, which is usually loudest at the left heart base (Kienle, 1998).

In severe cases of AS, concentric hypertrophy of the left ventricle occurs as a result of increased wall stress due to an increase in left ventricular pressure. In these dogs exertional syncope is a common clinical sign and they are at great risk of sudden death at a young age (Kienle, Thomas & Pion, 1994). The exact mechanisms behind the syncopes and the sudden death are not fully understood. One possible explanation is activation of ventricular baroreceptors by sudden exercise-induced increase in left ventricular systolic pressure, leading to acute reflex peripheral vasodilatation and bradycardia (Johnson, 1971; Mark *et al.*, 1973; Grech & Ramsdale, 1991). However, in dogs these changes have to be severe in order to cause fainting. A second possibility is myocardial ischemia due to the concentric hypertrophy, causing inadequate regional blood flow (Pyle *et al.*, 1973; Borkon *et al.*, 1982). Finally, recent studies on hypertrophic cardiomyopathy suggest that energy compromise is a contributing factor in the pathogenesis of that disease (Frey *et al.*, 2006; Ho & Seidman, 2006) and it is possible that energy consumption also plays an important role in the hypertrophied cardiac muscle of dogs with severe aortic stenosis. Myocardial ischemia and/or energy compromise could result in ventricular arrhythmias causing fainting or progressing into fatal ventricular fibrillation and sudden death in an exercising dog.

Treatment of dogs with moderate-severe AS is difficult. Surgical correction of the obstruction has not proved successful. Beta-adrenergic receptor blockers for protection of the myocardium against ischemia are recommended by some authors, but have not been evaluated in placebo-controlled clinical trials (Oyama, *et al.*, 2005).

Cardiac auscultation is used to identify the systolic ejection murmur created by the aortic stenosis. The murmur can be graphically displayed by use of phonocardiography (Kvart *et al.*, 1998; Kvart & Häggström, 2002), (Figure 1). Echocardiographic examination is used for confirmation of the disease. The subvalvular aortic stenosis can usually be identified by an experienced echocardiographer (Figure 2) and thickened aortic valves, indicative of valvular AS, can sometimes be visualized. Aortic insufficiency (AI), detected by colour Doppler imaging, is a common finding in dogs with subvalvular as well as valvular AS (Kienle, 1998). Severe stenotic lesions may involve the cranioventral leaflet of the mitral valve, causing mitral valve insufficiency (Oyama, *et al.*, 2005).

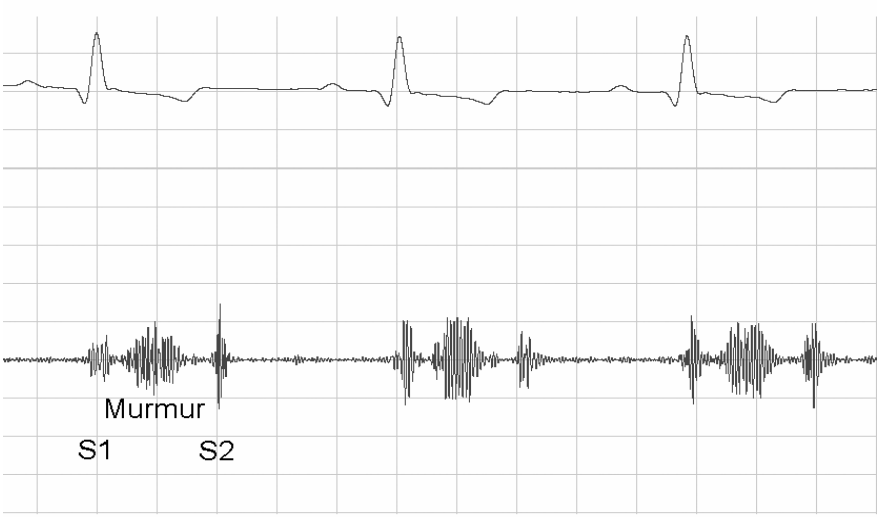

Figure 1. Phonocardiographic sound file from a boxer with moderate subvalvular aortic stenosis, showing the first heart sound, S1, the systolic ejection murmur and the second heart sound, S2. Note the crescendo-decrescendo shape of the murmur. A reference ECG, lead II, is shown on top. The sound file was obtained with maximal amplification at 200 Hz using the Welsh Allyn Meditron system.

In dogs with structural changes consistent with subvalvular and/or valvular AS, the aortic flow velocity is increased. Today, measurement of aortic flow velocity is performed by spectral continuous-wave Doppler echocardiography, which shows an excellent correlation with invasive measurements in dogs (Valdes-Cruz *et al.*, 1985; Lehmkuhl *et al.*, 1995). In order to get as straight an alignment as possible with the aortic flow, this measurement should preferably be made from the subcostal view (Lehmkuhl & Bonagura, 1994), (Figure 3).

Thoracic radiography is generally unrevealing in dogs with AS (O'Grady, *et al.*, 1989; Kienle, Thomas & Pion, 1994) and is rarely used for diagnosis of the disease. This technique has therefore not been used in the studies included in the thesis.

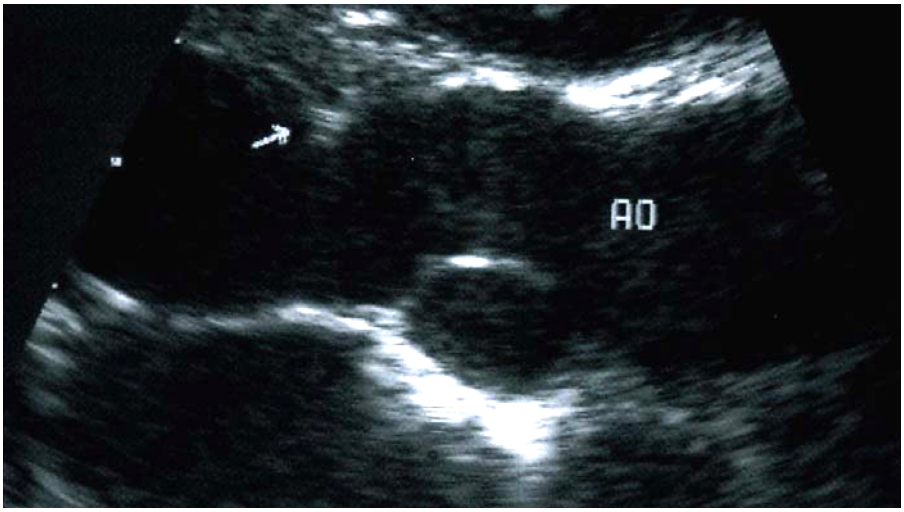

Figure 2. Two-dimensional echocardiographic image of the left ventricular outflow tract showing a subvalvular aortic stenosis (arrow) and the proximal part of the aorta (AO). The image was obtained from the right parasternal long-axis view.

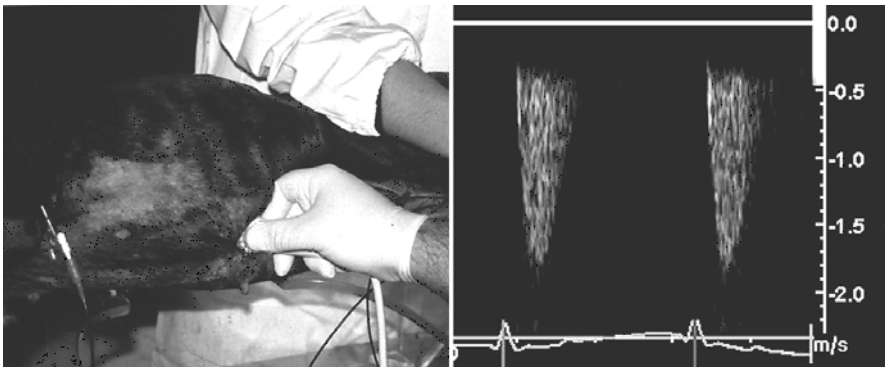

Figure 3. Echocardiographic Doppler examination in a boxer, showing the subcostal transducer placement at the posterior end of the sternum (left). The continuous-wave Doppler tracings (right) show a peak aortic flow velocity of 1.95 m/s. A reference ECG, lead II, is shown at the bottom of the screen.

Mild cases of AS are difficult to diagnose (Pyle, 2000; Bonagura, 2001; Oyama & Sisson, 2001; Oyama, *et al.*, 2005). Clinical signs are absent and the physical examination is unremarkable, apart from the presence of a systolic ejection murmur. By cardiac auscultation or standard phonocardiography, it is difficult to determine whether the murmur is innocent or caused by a mild form of AS. The echocardiographic examination is sometimes inconclusive in these dogs, with mildly increased aortic flow velocities and absence of a visual aortic stenosis.

A genetic basis for SAS, indicating a single major gene abnormality, has been demonstrated in the Newfoundland breed (Pyle, Patterson & Chacko, 1976; Harpster & Jones, 1991). A heritable basis for the disease is also strongly suspected in other affected breeds, such as the boxer, rottweiler, golden retriever and German shepherd (Jones, 1989; Harpster & Jones, 1991 ; Kienle, Thomas & Pion, 1994; Buchanan, 1999). Breeding experiments in specifically colony-bred Newfoundlands produced 30% off-spring with SAS. Since stenotic lesions were not detected by gross pathology in puppies less than 3 weeks and only mild forms of disease between 3 and 12 weeks of age, SAS was suggested to develop in the post-natal period, rather than being a true congenital defect (Pyle, Patterson & Chacko, 1976). Further prospective studies on the development and progression of aortic stenosis in other dog breeds have yet to be carried out.

The boxer breed - a short introduction

The boxer is a medium-sized, smooth-haired and sturdy dog with a square structure and heavy bones. It is a brachycephalic breed with a wide and very short face. The boxer is a bold dog, which is easy to train and often used for protection, companionship and serving. The boxer breed was developed in Germany with the first purebred dogs being born in 1898 (Tiback & Hedhammar, 1977).

The boxer is a popular breed world-wide. In Sweden, the number of boxers is approximately 4000 and around 550 puppies divided into 100 litters are registered each year.

Cardiovascular characteristics of boxer dogs

The prevalence of heart murmurs in the adult boxer population is between 50 and 80% (Luis Fuentes, 1993; Heiene *et al.*, 2000; Bussadori, Quintavalla & Capelli, 2001), a high figure compared to other breeds of dogs. The majority of the murmurs are mild systolic ejection murmurs. Some of the murmurs are caused by a mild form of AS (O'Grady, *et al.*, 1989; Luis Fuentes, 1993; Prosek, 2005), while the origin of the others is unclear. As previously described, innocent murmurs present in most other dog breeds diminish or resolve by the age of 6 months (Kvart & Häggström, 2002; Oyama, *et al.*, 2005; Prosek, 2005). However, this does not seem to happen in the majority of boxer dogs. Possible reasons for the high prevalence of heart murmurs in boxers are a smaller size of the left ventricular outflow tract and increased stroke volume in boxers compared to other dog breeds (Koplitz, Meurs & Bonagura, 2006). It is unknown whether a smaller size of the LVOT should be considered a specific breed feature in boxers or if it is associated with AS. The genetic association, if any, with subvalvular or valvular AS in boxers remains to be investigated.

Cardiovascular diagnostics in boxer dogs can be complicated. Being a brachycephalic breed, boxers have narrow upper airways, which causes extra respiratory noise, thus making cardiac auscultation difficult. The boxer is an active and playful dog, and is easily excited. As a result, detection and grading of low intensity murmurs in boxers may be difficult. Additionally, variations in murmur intensity as well as aortic flow velocity have been observed in boxers, possibly

depending on the level of sympathetic stimulation of the dog being examined (Bonagura & Luis Fuentes, 2000; Heiene, *et al.*, 2000).

Phonocardiography can be used as an aid in evaluation of systolic ejection murmurs in boxers (Kvart, *et al.*, 1998), although using standard phonocardiography, differentiation between a low intensity ejection murmur of uncertain origin and a murmur caused by mild AS is difficult (Figure 4).

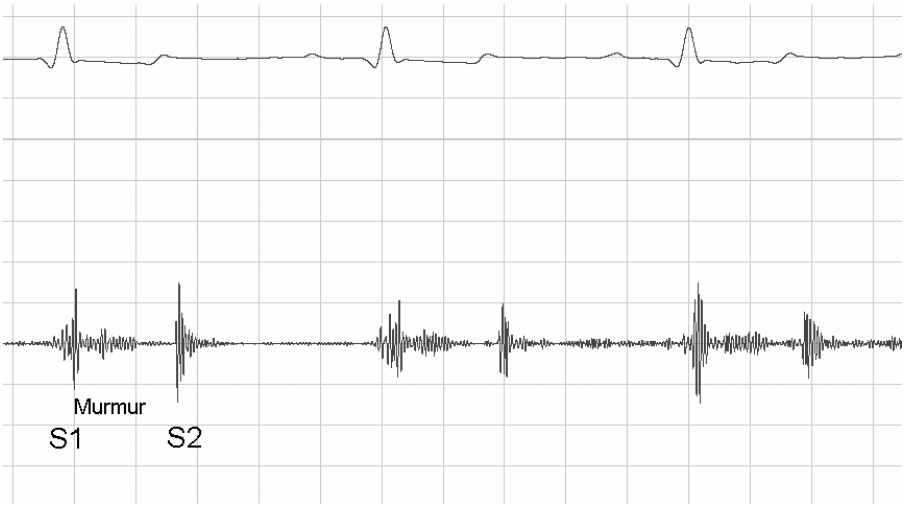

Figure 4. Phonocardiographic sound file showing a low intensity systolic ejection murmur in a 6 month old boxer without structural changes consistent with AS on 2D echocardiography. The murmur was graded I/VI using an acoustic and II/VI using an electronic stethoscope. The aortic flow velocity measured by subcostal continuous-wave Doppler was 1.95 m/s. The sound file was obtained with maximal amplification at 200 Hz, using the Welsh Allyn Meditron system. A reference ECG, lead II, is shown on top.
S1=first heart sound, S2=second heart sound

Echocardiography, including 2D and Doppler examinations, is considered the gold standard for diagnosis of most cardiac diseases, but in boxers with low intensity ejection murmurs it is sometimes inconclusive, both in puppies and in adult dogs. Spectral Doppler echocardiography using the subcostal transducer location may be difficult to perform in some boxer dogs, due to the long distance from the transducer to the point of measurement. In adult boxer dogs this distance is usually around 20 cm. In addition, boxer dogs have narrow, deep chests rendering the examination even more difficult. Development and improvement of echocardiographic methods, as well as the search for new and more reliable methods for murmur evaluation in order to differentiate between innocent and pathological murmurs, are therefore considered desirable.

The focus of this thesis has been studies of systolic ejection murmurs and their underlying causes. Since there is a high prevalence of heart murmurs in the boxer breed and these murmurs are present in boxers with, as well as without, structural changes consistent with subvalvular or valvular AS on 2D echocardiography, this breed was chosen for the studies. The suggestion that AS is not a truly congenital disease in Newfoundland dogs (Pyle, Patterson & Chacko, 1976) prompts

investigation of the cardiac development in boxers during their first year of life. Investigations of growing boxers of which some would remain healthy and some might develop AS, would offer a good possibility to obtain increased knowledge of suitable methods for evaluation of heart murmurs.

Aims of the thesis

The objectives of this thesis are as follows:

- To objectively estimate inter-observer variation in cardiac auscultation of boxers with low intensity systolic ejection murmurs.
- To investigate if exercise and noise stimulation affects the aortic flow velocity and the grading of systolic ejection murmurs in boxers.
- To investigate whether contrast echocardiography could enhance the subcostal Doppler signal and allow assessment of myocardial blood flow in boxer dogs with or without AS.
- To investigate whether the standard investigation methods used to diagnose systolic ejection murmurs can be improved by using time-frequency and complexity analyses.
- To study the progression of systolic ejection murmurs, development of the LVOT and flow velocities in the great arteries in growing boxer dogs.

Materials and methods

Animals

A total of 85 pure-bred boxer dogs, 50 females and 35 males have been examined in the studies performed in this thesis. In paper I, twenty-seven boxers with a mean age of 2.7 ± 2.1 years were studied. Paper II included twenty-nine boxer dogs with a mean age of 3.0 ± 2.3 years. In paper III two experiments were performed. The first experiment included 16 boxers examined twice, at 7 weeks and 12 months of age. In the second experiment of paper III, the results of the 12-month examination of the dogs in experiment 1 were compared to a group of 11 boxers with AS aged 3.7 ± 2.8 years. In paper IV, a cohort of sixteen boxer dogs was studied prospectively during their first year of life, with a total of 6 examinations per dog. Fourteen of these dogs had been included in the investigations of paper III and two new dogs were added. Hence, the total number of examinations included in this thesis was 161.

All dogs included in the investigations were privately owned and only spent time at the clinic during examinations. Dogs with a case-history of clinically

significant ongoing organ-related or systemic disease were not included in the investigations. Dogs with cardiac diseases other than AS were excluded from the studies. All examinations were performed on unsedated dogs.

The investigations took place in three different countries, Great Britain (paper I), Italy (paper II) and Sweden (papers III and IV) and the dogs came from different breeders. Dogs for the investigations carried out in papers III and IV were recruited in cooperation with the Swedish Boxer Club.

Standard methods of examination

The dogs were brought to the examinations by their owners. For the investigations presented in papers I and II, the dogs were left at the clinic during the procedures and were allowed home later that day. During the investigations leading to papers III and IV, the owners stayed with their dogs.

An overview of the procedure of examination is given in table 2. Upon arrival the dogs were weighed and the nutritional status of the dogs was assessed. Anamnestic information on each dog was obtained from the owner during a short interview and a physical examination was performed. Detailed description of the cardiovascular diagnostic techniques is given in the text below. The dogs were allowed to rest between the different parts of the cardiac examination.

Table 2. *Overview of the procedures for the examinations of the dogs in the four papers included in the thesis.*

Procedure	Paper I	Paper II	Paper III	Paper IV
Interview with owner	X	X	X	X
Physical examination	X	X	X	X
Cardiac auscultation	X	X	X	X
ECG examination	X	X	X	X
PCG examination	X	X	X	X
Echocardiographic examination	X	X	X	X
Blood analysis of troponin I*		X		
Blood analysis of haemoglobin			X	X

* Analysed by IMMULITE Troponin I (Diagnostic Products Corp., Los Angeles, CA, USA)

Cardiac auscultation

The cardiac auscultation took place in a quiet examination room with the dog in a standing position. If a murmur was detected, the intensity was graded I-VI according to an established system (Gompf, 1988) and the timing in the cardiac cycle, as well as point of maximal intensity of the murmur were noted. Absence of heart murmur was graded as 0.

In paper I, dogs with low intensity ejection murmurs were auscultated by six independent examiners with different levels of experience in order to study the inter-observer variation. The findings of this study showed a high variability of low intensity ejection murmurs and the importance of experience in auscultation of these murmurs. Hence, dogs with low intensity ejection murmurs were thereafter auscultated by two experienced examiners and the degree of heart murmur was decided by taking the average of the grades assigned by the two independent examiners (papers II, III and IV).

Acoustic stethoscopes (Rappaport-Sprague, Supraphone, Wihlem Hasselmeyer Medical Development Invest. GmBH, Buchen, Germany) were used for cardiac auscultation in papers I and II. In paper III Welsh Allyn Meditron sensor-based electronic stethoscopes (Meditron ASA, c/o Medi-Stim ASA, Oslo, Norway) were used and in paper IV both of the stethoscopes described above were used. The electronic stethoscope allows regulation of volume and frequency settings, as well as allowing several observers to listen simultaneously by connection of additional earphones to a distributing unit (The Meditron Distributor).

PCG and ECG examination

Phonocardiographic (PCG) recordings were obtained in a quiet examination room with the dog in a standing position. In papers I and II, phonocardiograms were recorded using a handheld piezo-electric phonocardiographic microphone (Hellige GmbH, Freiburg, Germany) connected to a Siemens Mingograph Minor 3/Phono amplifier (Siemens-Elema AB, Solna, Sweden), (Häggström, Kvart & Hansson, 1995; Heiene*, et al.*, 2000). In paper III, the Welsh Allyn Meditron electronic stethoscope connected to a Dell Latitude D800 laptop (Dell computer corp., Limerick, Ireland) equipped with the Meditron Analyzer software program, version 4.0V (Meditron ASA, c/o Medi-Stim ASA, Oslo, Norway) was used for PCG recordings. Both PCG systems provide a reference ECG in lead II. For all recordings the microphone (papers I and II) or chest piece of the stethoscope (paper III) was placed over the aortic area at the left heart base. The PCG recordings presented in papers I and II were evaluated blindly by one examiner, according to a manual protocol previously described (Häggström, Kvart & Hansson, 1995; Heiene*, et al.*, 2000) and the dogs were grouped according to the severity of the phonocardiographic changes (Kvart*, et al.*, 1998). In paper III, PCG recordings were evaluated by a new computerised method, as described below.

The heart rhythm was evaluated on ECG lead II provided with the PCGs in papers I-III. In paper IV, evaluation of heart rhythm was made on ECGs obtained using a Nihon Kohden Cardiofax GEM ECG machine, model 9020K (Nihon Kohden Corp., Tokyo, Japan).

Echocardiographic examination

The echocardiographic examinations took place in a quiet examination room with the dog in right and left lateral recumbency. Complete echocardiographic examinations were performed with standardised imaging planes (Thomas *et al.*, 1993). Continuous ECG monitoring was used on all dogs. Pulsed wave Doppler was used for measurement of pulmonic flow velocity. Aortic flow velocity was measured by continuous-wave Doppler using the subcostal transducer location (Lehmkuhl & Bonagura, 1994). M-mode measurements of the left ventricle were made using standard techniques. The sizes of the aorta and left atrium were measured on the 2D echocardiogram (Hansson *et al.*, 2002). The mitral, tricuspid, aortic and pulmonic valves were evaluated for presence of valve leakage using colour flow Doppler.

Esaote and GE ultrasound systems were used for the studies, an Esaote Sim 7000 Challenge machine in paper I, an Esaote Biomedica Megas 449 Xa Plus Multigraph in paper II (both from Esaote Biomedica, Firenze, Italy) and a GE Vivid 3 ultrasound machine (General Electric Company, Stockholm, Sweden) in papers III and IV.

Studies of inter-observer variation and stress testing

Inter-observer variation in cardiac auscultation (Paper I)

Due to the well-known difficulties in cardiac auscultation of boxer dogs, we were interested in studying inter-observer variation between examiners with different levels of experience in cardiac auscultation. Six examiners, two with extensive experience in small animal cardiology, two with approximately one year post-graduate experience in small animal cardiology and two fourth year students independently auscultated dogs with low intensity ejection murmurs, first at rest and then after stress-testing. The examiner recorded the presence of any systolic heart murmur, its intensity (I-VI), character and point of maximal intensity as well as the presence of any diastolic heart murmur. The heart rate at rest was recorded.

Stress testing (Papers I and IV)

In papers I and IV, dogs were made to run a short distance in order to investigate the effects of stress testing on cardiac auscultation. Immediately after exercise, the dog was auscultated and heart rate as well as presence and intensity of heart murmur were recorded. In paper I, each dog was made to run 6 times in order for all examiners to auscultate following exercise. In study IV, each dog ran once at the end of each visit and two examiners auscultated simultaneously using a single Welsh Allyn Meditron electronic stethoscope with an additional earphone. For practical reasons it was not possible to exercise the small puppies during the first visit in paper IV. The stress testing on this occasion therefore consisted of a quick lifting manoeuvre (Pedersen *et al.*, 1999). The puppy was standing on the examination table, then lifted quickly down to the floor and up again. The manoeuvre was repeated twice and was then immediately followed by simultaneous auscultation by two examiners using the electronic stethoscope.

Stress testing for the PCG and echocardiography studies reported in paper I, was performed by exposing the dogs to two different noise stimuli; first a tape-recording of a barking dog and, secondly, a squeaky toy. The stress testing for PCG was performed in standing dogs, following the initial recording at rest. The microphone was kept over the aortic area and the recording continued while the dog was exposed to the noise stimuli. The recordings were later evaluated blindly by one examiner, according to a manual protocol previously described (Häggström, Kvart & Hansson, 1995; Heiene, *et al.*, 2000). During the echocardiographic examination, stress testing was performed while measuring aortic flow velocity by continuous-wave Doppler from the subcostal transducer location, with the dog in right lateral recumbency. Following measurement at rest, the transducer was kept in the same position while the dog was exposed to the two noise stimuli. Time was allowed for the aortic flow velocity to return to resting

level between examinations. The examinations were recorded on videotapes and later reviewed blindly by one examiner.

Studies of murmur progression and development of the left ventricular outflow tract *(Paper IV)*

Due to the high prevalence of low intensity ejection murmurs in the adult boxer population (Luis Fuentes, 1993; Heiene*, et al.*, 2000; Bussadori, Quintavalla & Capelli, 2001), we were interested in studying the progression of heart murmurs, development of the LVOT and flow velocities in the great arteries during the first year of life in boxers. Matched pairs of boxers, one puppy with a low intensity ejection murmur and one without, from 8 different litters were therefore selected. None of the puppies had structural evidence of heart disease at inclusion at 7 weeks of age. The dogs were studied prospectively with examinations at 7 weeks and 3, 4, 6, 9 and 12 months of age. The dog in each litter which did not have a heart murmur at inclusion was considered as belonging to the non-murmur group, while the litter mate with a low intensity ejection murmur at inclusion belonged to the murmur group.

At all examinations, cardiac auscultation at rest and after stress testing was performed. Besides routine echocardiographic examination, specific evaluation of the LVOT was performed at each examination. In the right and left long-axis views, the subvalvular, valvular and supravalvular regions of the aorta were inspected for abnormal morphology. In each dog, the image frame with the highest quality from the right parasternal long-axis view was selected. On this frame, measurements of the subvalvular and valvular diameters in early diastole were made. The blood flow in the LVOT was screened using colour flow Doppler from the right and left parasternal long-axis views.

In order to investigate the smallest size of the LVOT, the effective orifice diameter (EOD) was calculated (Bélanger *et al.*, 2001). The subvalvular and valvular diameters, as well as the EOD, were indexed to body surface area.

Changes in body size variables and echocardiographic data over time were also evaluated and compared between the non-murmur and murmur groups.

Evaluation of complementary methods

Using standard methodology, basic diagnostic information on structural as well as hemodynamic features of the heart in boxer dogs with systolic ejection murmurs was obtained. In order to investigate whether heart murmurs in boxers contain further diagnostic information and if the echocardiographic evaluation could be improved in these dogs, three methods were investigated: contrast echocardiography and time-frequency analysis of heart murmurs - both of which have been used in humans with AS (Almeida *et al.*, 2002; Smith *et al.*, 2004; Tavel & Katz, 2005) – and complexity analysis using the fractal dimension of the sound signal, which is a new method in heart murmur evaluation.

Contrast echocardiography (Paper II)

In order to study whether contrast echocardiography could enhance the subcostal Doppler signal for aortic flow measurements and improve assessment of myocardial blood flow by myocardial opacification, the contrast agent Optison (Mallinckrodt Medical GmbH, Hennef, Germany) was evaluated in boxer dogs with and without structural changes consistent with AS on 2D echocardiography. Optison is a second-generation, intravenous contrast agent, consisting of microspheres of heat treated human albumin containing octoflouropropane, suspended in a 1% human albumin solution. The contrast study was performed with a special focus on the Doppler examination of aortic flow velocity.

The dogs were divided into two groups, which received an intravenous injection of either a low dose of Optison (0.025-0.1 ml) or a slightly higher dose (0.2 ml). The examinations were recorded on super VHS videotapes. The subcostal Doppler signal was later evaluated qualitatively before and after contrast, the duration of contrast enhancement was measured and the 2D myocardial image quality was assessed by three independent observers.

In order to monitor the health status and check for any adverse side reactions to Optison in the dogs, a questionnaire was sent to all dog owners 6 months after participation in the study.

Time-frequency and complexity analyses of heart murmurs (Paper III)

We were interested in differentiating ejection murmurs in boxers with and without aortic stenosis. PCG recordings were therefore obtained from boxer dogs with and without structural changes consistent with AS, identified by 2D echocardiography, using the Welsh Allyn Meditron electronic stethoscope connected to a Dell Latitude D800 laptop equipped with the Meditron Analyzer software program, version 4.0V. Analysis of the PCG recordings were made using MATLAB 7.04 (The MathWorks, Inc., Natick, MA, USA). All recorded PCG signals were manually segmented by one examiner and only the systolic part of the signal was used for the analysis.

Time-frequency representation of sound includes frequency on the vertical axis, time on the horizontal axis and intensity by use of colours (see cover illustration). This way, TFP can be used as a means of investigating how the frequency content of a signal varies over time. Inspired by the work performed in human cardiac patients (Tavel & Katz, 2005), two parameters were defined to characterize the murmur. The first parameter registers the maximal frequency of the murmur and the second parameter is determined as the duration of murmur frequency exceeding 200 Hz.

Another means of analysing sound is by investigating the complexity of the sound signal. Normal heart sounds have a defined structure, murmurs are more complicated in nature and background noise has no structure at all (Nigam & Priemer, 2005). The fractal dimension of a signal is one way to measure complexity (Sprott, 2003; Kantz & Schreiber, 2004; Kinsner *et al.*, 2006). In order to study if flow behaviour could be characterized by the fractal dimension of the ejection murmur, two different methods were evaluated. Firstly, the variance fractal dimension (VFD) was investigated (Kinsner, *et al.*, 2006). VFD tries to

estimate the fractal dimension by operating directly on the waveform of the signal. This simplification does, however, make the method unreliable in the presence of background noise if the amplitude of the source signal varies significantly between dogs, as is the case when investigating various degrees of stenosis. To avoid this problem, VFD had to be abandoned and replaced by the correlation dimension (T_2), (Sprott, 2003), which operates in a reconstructed state space of the signal. In this way, the above mentioned problem can be avoided. Hence, in order to study if it was possible to use the fractal dimension of the murmur for differentiation between dogs with or without structural changes consistent with AS on 2D echocardiography, T_2 was calculated.

Results

Overall description of clinical status *(Papers I-IV)*

In all dogs, the nutritional status was assessed as within normal limits. In dogs without structural changes consistent with AS, no clinical signs of reduced physical performance or fainting had been observed by the owners and no pathologic arrhythmia was detected on ECG recordings or during the echocardiographic examinations. The haemoglobin concentration in dogs with low intensity murmurs but no structural changes consistent with AS, was within normal limits in all dogs at 12 months of age (papers III and IV). Hence, decreased blood viscosity due to anaemia could be excluded as a cause of the low intensity ejection murmurs at 12 months of age. The concentration of cardiac troponin I was within normal limits in all, but three dogs, examined in paper II. In the remaining dogs there was a very mild increase in troponin I, compared to the upper limit recommended by the laboratory.

In two dogs with severe subvalvular AS (papers II and III), their owners reported occurrences of occasional fainting during exercise. In one of these dogs, frequent ventricular premature contractions were present on the ECG. No pathologic arrhythmia was detected on ECGs from dogs with mild-moderate AS.

All dogs included in the studies of paper IV showed a normal growth pattern, as assessed by measurements of bodyweight, thoracic circumference and femoral length.

Inter-observer variation in cardiac auscultation *(Paper I)*

In the 27 examined dogs with or without SAS, all six examiners graded the murmurs at rest between 0 and III/VI. The total number of systolic heart murmurs identified by the different auscultators varied between 15 and 22. The majority of the murmurs had their point of maximal intensity over the aortic area at the left heart base and were characterised as soft and/or crescendo/decrescendo by the examiners. They could thus be described as low intensity systolic ejection murmurs. Only one observation of a diastolic murmur was made, by one of the low-experienced examiners.

The agreement in murmur grading was only good between the two most experienced examiners with a weighted kappa value (κ) of 0.75. The low and medium experienced examiners showed poor to moderate agreement with the most experienced examiners, with weighted κ-values of 0.16-0.55 (Weighted κ-values are graded on a scale from 0-1 (Altman, 1991)).

Stress testing

Effects of stress testing in cardiac auscultation (Papers I and IV)

In paper I, the examiners detected systolic ejection murmurs in 20-25 of the 27 dogs after exercise, with the highest number of murmurs being detected by the most experienced examiners. Heart rates were significantly higher after stress testing and the increased heart rate lasted for 30-40 seconds post-exercise. After stress testing, observed murmur grades increased significantly for 5 out of 6 examiners. The range of murmur grades was 0 to IV/VI. An increase in murmur grading after exercise was detected in a higher percentage of dogs by the two most experienced examiners, compared to examiners with moderate and low experience. The two most experienced examiners detected murmurs in 93% of the dogs after stress testing, compared to 78% at rest. A large proportion of the low intensity murmurs increased by at least one grade after exercise, with the highest increase in dogs with an initial grade I murmur. The inter-observer agreement decreased remarkably after exercise compared to rest, with only poor to fair agreement between all examiners.

In paper IV, cardiac auscultation was performed by two examiners experienced in auscultation of boxer dogs. Boxer dogs, none of which had structural changes consistent with AS at inclusion, were examined 6 times during their first year of life and auscultation at rest as well as after stress testing was performed on each occasion. Whilst 50% of the dogs did not present with a heart murmur at inclusion in the study at 7 weeks of age, all dogs displayed a murmur after stress testing on several or all occasions. Overall, murmurs were detected after stress testing in 96% of the 90 observations performed during the study period. The range of heart murmur grades after stress testing was 0-II/VI, with the majority being graded II/VI.

Effects of stress testing on phonocardiographic and echocardiographic findings (Paper I)

Stress testing during phonocardiographic and echocardiographic examinations was performed by exposing the dogs to two different noise stimuli. The first was a tape recording of a barking dog and the second a squeaky toy. After the second stress test, murmur durations measured on PCG recordings increased significantly compared to rest, whereas there was only a tendency ($p=0.057$) after the first stress test. The mean aortic flow velocity, measured by continuous wave Doppler echocardiography, increased significantly after the first stress test, but not after the second.

Progression of systolic ejection murmurs *(Paper IV)*

In most of the 16 dogs there was a considerable variation both in the presence and intensity of heart murmurs detected by cardiac auscultation at rest, during the growth period. In all dogs which presented without a murmur at inclusion (the non-murmur group), a low intensity murmur was detected on at least one occasion during the study period. Only one of the dogs in the group presenting with a low intensity ejection murmur at inclusion (the murmur group) had the same murmur grade at every examination (grade II). The range of heart murmur grades in the non-murmur group, as well as in the murmur group, was 0-II/VI. The point of maximal intensity of all murmurs detected at rest during the study period was at the left heart base and all murmurs were classified as low intensity ejection murmurs.

At 12 months of age, the end-point of the study, five of the eight dogs in the non-murmur group and four of the eight dogs in the murmur group, presented with a low intensity ejection murmur.

The left ventricular outflow tract

Development of the left ventricular outflow tract (Paper IV)

None of the 16 dogs developed structural changes consistent with subvalvular aortic stenosis on 2D echocardiography during their first year of life. There were no differences in LVOT subvalvular diameter between the non-murmur group and the murmur group. Neither did the LVOT valvular diameter differ between the groups. There were no significant differences between LVOT diameters (subvalvular, valvular and effective orifice diameter) of the 16 dogs at any of the examinations. Neither did the diameters indexed to body surface area differ significantly.

Three of the dogs in the murmur group developed aortic insufficiency (AI) during the study period. In two of the dogs, the AI was mild, and in one the AI was moderate at 12 months of age. The left ventricular diastolic diameter (LVDd) at the end-point of the study was significantly larger in the murmur group, compared to the non-murmur group. None of the other echocardiographic data or body size variables evaluated over time differed significantly between the two groups.

Comparison between dogs with and without structural changes in the left ventricular outflow tract (Papers I-IV)

Data on heart murmur grades and peak aortic flow velocities of all dogs are summarized in table 3. The total number of dogs without structural changes consistent with subvalvular or valvular AS on 2D echocardiography was 61. Evidence of structural changes consistent with AS was detected in 24 dogs, 18 of which were subvalvular and 6 valvular. In paper I, the SAS were all mild (peak aortic flow velocity $\leq$3.2 m/s). In paper II, 2 dogs had mild AS and 5 dogs had moderate to severe AS (peak aortic flow velocity >3.2 m/s), whereas 5 dogs had mild AS and 6 dogs moderate to severe AS in paper III.

Auscultatory murmur grades ranged between 0-III/VI in dogs without structural changes consistent with subvalvular or valvular AS on 2D echocardiography and I-VI in dogs with structural changes consistent with AS. The murmur grades, as well as the aortic flow velocities, were significantly higher in dogs with structural changes consistent with AS, compared to dogs without (papers I-III). Peak aortic flow velocities ranged between 1.19 and 2.53 m/s in dogs without structural changes consistent with AS on 2D echocardiography, and between 1.91 and 6.78 m/s in dogs with structural evidence of AS.

In paper I, thirteen dogs (48%) had mild aortic valve thickening and five dogs (19%) had mild aortic insufficiency (AI). There was no association between the mild aortic valve thickening and aortic flow velocity. Hence, these dogs were not considered affected by AS at the time of that study. However, considering that dogs with AI had significantly higher aortic flow velocities at rest as well as after both stress tests, mild valvular aortic stenosis can not be excluded in dogs with mild valve thickening and AI. On the other hand, three of the five dogs with AI also had mild SAS, which might explain the higher aortic flow velocity. Five dogs (19%) had mild mitral insufficiency, which was not correlated to initial murmur grading, aortic flow velocity or murmur duration.

In paper IV, two of the three dogs which developed AI before the age of 12 months, displayed slightly increased aortic flow velocities on 3 occasions during the study period. Whilst numerically higher, flow velocities were not significantly higher in these dogs compared to dogs without AI.

If dogs with structural changes consistent with AS and dogs with AI are excluded, the range of peak aortic flow velocity was 1.38-2.28 m/s in paper I, 1.52-2.09 m/s in paper III and 1.43-2.11 m/s in paper IV.

Table 3. *Results from cardiac auscultation and echocardiographic examinations of dogs with and without structural changes consistent with aortic stenosis (AS) on 2D echocardiography. Peak aortic flow velocities (Vmax aorta) are reported at baseline, i.e. resting values in paper I and values before contrast injection in paper II. n=number of dogs. Exp.=experiment*

		No structural changes consistent with AS			**Structural changes consistent with AS**			
	n	n	Murmur grade (0-VI)	Vmax aorta (m/s) Mean ± SD (range)	n	Murmur grade (0-VI)	Vmax aorta (m/s) Mean ± SD (range)	Comments
Paper I	27	21	0-III	1.74 ± 0.05 (1.38-2.35)	6	I-III	2.15 ± 0.21 (1.91-2.52)	6 SAS
Paper II	29	22	0-II	1.90 ± 0.30 (1.19-2.42)	7	III-VI	4.53 ± 1.59 (2.45-6.78)	5 SAS 2 valvular AS
Paper III								
Exp. 1	16	16*	I-II	1.76 ± 0.15 (1.43-1.97)				
Exp. 2	27	16*	I-II	1.83 ± 0.24 (1.52-2.41)	11	II-V	3.84 ± 1.08 (2.40-5.50)	7 SAS 4 valvular AS
Paper IV	16	16#	0-II	1.83 ± 0.22 (1.43-2.53)	0			

* In paper III, the same 16 dogs were examined twice. Figures are given from the examination at 7 weeks in experiment 1 and the examination at 12 months in experiment 2. # Means of all 6 examinations in the 16 dogs.

Contrast echocardiography *(Paper II)*

Doppler signals of aortic flow obtained from the subcostal view at baseline were judged as weak in 66% of the 29 dogs. Signal enhancement after contrast injection with Optison was seen in all dogs.

The peak aortic flow velocity in all dogs increased slightly from 2.58 ± 1.42 m/s before contrast to 2.71 ± 1.54 m/s after contrast. Comparing the dose groups, the highest percentage of Doppler examinations showing optimal signal enhancement was obtained in the low dose group (0.025-0.1 ml), while a substantial number of Doppler examinations using the high dose (0.2 ml) of Optison resulted in excessive signal enhancement with saturation artefacts. The mean duration of Doppler signal enhancement was 4½ minutes (range 3-5½) in the high dose group and 4 minutes (range 2-4½) in the low dose group, allowing sufficient time for evaluation and measurements using both doses.

Before contrast, the 2D myocardial image quality was judged as good in 46%, intermediate in 53% and poor in 1% of the observations made by three independent observers. The inter-observer agreement between the two blinded observers was good with a weighted κ-value of 0.67. Comparing assessments made before and after contrast, none of the three observers found significant differences in 2D myocardial image quality both when looking at all dogs taken together and when looking at the two dose groups. Hence, contrast echocardiography using Optison could not be used for evaluation of myocardial blood flow, with the present technique.

No acute adverse side effects were seen during Optison application or within several hours thereafter. The questionnaire investigating the health status of the dogs during the six months following the study resulted in one report of a dog which had died due to gastric dilatation volvulus. None of the other dogs had shown signs of any disease or adverse side effects obvious to the owners.

Time-frequency and complexity patterns of systolic ejection murmurs *(Paper III)*

Using standard phonocardiography, significant correlation was found between auscultatory murmur grades and severity of phonocardiographic changes for the two most experienced, as well as one of the moderately experienced, examiners in paper I. The severity of phonocardiographic changes also correlated well with auscultatory results in paper II. In order to investigate whether further diagnostic information of heart murmurs could be found in boxer dogs with or without structural changes consistent with AS on 2D echocardiography, PCG recordings obtained from 27 dogs were evaluated by TFP and T_2 analyses. The murmurs ranged in intensity between I and V/VI and they were all classified as systolic ejection murmurs.

In experiment I, comprising dogs with low intensity ejection murmurs but no structural changes consistent with AS, TFP and T_2 analyses of PCG recordings showed no significant differences in maximal frequency, duration of murmur frequency >200 Hz and T_2 of the murmur between the examinations at 7 weeks

and 12 months of age. In addition, neither the auscultatory murmur grade nor the peak aortic flow velocity differed between the two examinations.

In experiment 2, in which results of the TFP and T_2 analyses were compared between dogs with, and dogs without, structural changes consistent with AS, the values of maximal frequency, duration >200 Hz as well as T_2 of the murmur were all significantly higher in dogs with AS compared to dogs without. When the dogs were further divided into four subgroups (based on peak aortic flow velocity), the maximal frequency of the murmur was significantly higher in dogs with moderate to severe AS compared to dogs without AS but with slightly increased peak aortic flow velocities. Comparing the two subgroups without AS, dogs with lower aortic flow velocities did, however, have higher maximal murmur frequency than dogs with slightly increased peak aortic flow velocities, a somewhat surprising finding. The duration of murmur frequency >200 Hz was significantly higher in dogs with moderate to severe AS compared to both subgroups without AS. It was also significantly higher in dogs with mild AS compared to both subgroups without AS. T_2 of the murmur was significantly higher in dogs with moderate to severe AS compared to both subgroups without AS. T_2 was also significantly higher in dogs with mild AS compared to dogs without structural changes consistent with AS and with low aortic flow velocities. The higher T_2 values indicate a higher complexity of systolic ejection murmurs in dogs with AS compared to dogs without.

Pair-wise comparison between the three methods (maximal frequency vs. duration of murmur frequency >200 Hz, maximal frequency vs. T_2 and duration of murmur frequency >200 Hz vs. T_2) gave significant models for separation between dogs with and without AS in all three cases. The correlation was 0.81, 0.71 and 0.82 for the three pairs, respectively. The sensitivities were 87.5%, 87.5% and 93.8% respectively and the specificities were 81.8% for all three pairs. Hence, optimal separation between dogs with and without structural changes consistent with AS on 2D echocardiography was obtained by combining duration of murmur frequency >200 Hz with T_2 analysis.

When comparing the seven dogs with subvalvular AS to the four dogs with valvular AS, no significant difference was found in peak aortic flow velocity between the groups. The severity of the stenoses was therefore considered equal between the groups. Neither the maximal frequency, nor the duration >200 Hz of the murmur differed significantly between the groups. T_2 of the murmur was, however, significantly higher in dogs with subvalvular AS compared to dogs with valvular AS, indicating a higher complexity of murmurs produced by a subvalvular obstruction compared to a valvular one.

Discussion

This thesis focuses on the boxer breed due to its high prevalence of heart murmurs (Luis Fuentes, 1993; Heiene*, et al.*, 2000; Bussadori, Quintavalla & Capelli, 2001) and increased relative risk of aortic stenosis (O'Grady*, et al.*, 1989; Luis Fuentes, 1993; Kienle, Thomas & Pion, 1994; Tidholm, 1997; Buchanan, 1999; Bussadori, Quintavalla & Capelli, 2001). A smaller size of the LVOT in boxers, compared to other breeds, could possibly be the cause of the low intensity ejection murmurs found in the majority of dogs without structural changes consistent with AS (Koplitz, Meurs & Bonagura, 2006). It is as yet unknown whether there is a genetic connection between a potentially smaller size of the LVOT and the development of subvalvular or valvular stenosis, which makes genetic counselling difficult. The difficulties in diagnosing the mildest cases of AS (Pyle, 2000; Oyama*, et al.*, 2005) render breeding counselling even more complicated.

For the owner of an asymptomatic puppy or adult dog with a murmur, it is important to know whether the murmur is potentially dangerous. When examining dogs for health certificates for sale or insurance purposes, the examining veterinarian has to assess the significance of a detected murmur. Prospective studies on cardiac development in boxer puppies during their first year of life, combined with investigations in adult boxer dogs with and without AS, enabled us to study various aspects of heart murmurs in dogs.

Privately-owned boxers from many different kennels were used in the studies presented in this thesis, which allowed the study of naturally occurring heart murmurs in dogs with or without spontaneous LVOT obstruction. This way, possible causes for the low intensity ejection murmurs could be investigated and the results of the investigations could potentially become clinically valuable, a strategy which has previously proved successful in cardiovascular research (Leatham, 1987).

Heart murmur characteristics in boxer dogs

Several studies have shown a high prevalence of systolic ejection murmurs in boxer dogs. An overview of the studies is given in table 4.

Table 4. *Percentage of systolic ejection murmurs detected by cardiac auscultation in boxer dogs at surveys in five European countries.* *Murmur grading after stress testing.*

Country	Nr of dogs	Murmurs	Reference
Italy	500	53%	Bussadori *et al*. 2001
Great Britain	319	50%	Luis Fuentes 1993
Norway/Sweden	231	77%	Heiene *et al*. 2000
Denmark	55	73%*	Linde & Koch 2006
Sweden	59	71%	Höglund *et al*. (unpublished data)

In the studies by Bussadori *et al*. (2001), Heiene *et al*. (2000) and Linde & Koch (2006), only adult dogs over one year of age were included. The examinations in Great Britain by Luis Fuentes (1993) were carried out at dog shows and the age of

the dogs is not given. The unpublished data by Höglund *et al*. comprises of ten litters of boxer puppies examined at 7-8 week of age in order to select dogs for the study presented in paper IV of this thesis. The incidence of systolic ejection murmurs ranged between 33% in the least affected litter to 100% in the most affected litter. The majority of the murmurs (range 66-98%) reported in the surveys of table 3 were of low intensity, *i.e.* graded I-II/VI. These studies were all performed on groups of dogs which were not randomly selected and, as a result, they may not be representative of the prevalence in the general boxer population.

Variation in murmur intensity

In the study by Heiene *et al*. (2000), variation in the intensity of heart murmurs during cardiac auscultation and phonocardiographic examination at rest was observed in 55% of 231 adult dogs, primarily dogs with grade I/VI murmurs. In another study, 48 healthy adult boxers examined by auscultation and echocardiography were re-examined one year after the initial examination. The results showed that 15% of the dogs without a murmur on initial examination presented with a low intensity murmur on follow-up, whereas a low intensity murmur (observed at the initial investigation) had disappeared in 45% of the dogs (Koplitz *et al.*, 2003). In paper IV of the present thesis, frequent examinations revealed a large variability in both the presence and intensity of low intensity murmurs during the first year of life in boxers. Whilst 8 of the 16 puppies did not present with a murmur at inclusion, low intensity ejection murmurs were detected in all dogs at rest on at least one occasion during the study period. One proposed reason for the variability in murmur intensity has been variations in sympathetic stimulation, but this has not previously been studied in boxers. The investigations of papers I and IV revealed a significant increase in the percentage of systolic ejection murmurs as well as their intensity after exercise. Even though the type of exercise differed (in paper I, adult dogs ran 30-50 meters on a leash, whereas the young dogs studied in paper IV were placed in an enclosed corridor and encouraged to run and play for approximately 3 minutes) the results were similar. Using cardiac auscultation, heart murmurs were detected after exercise in 93 and 96% of the observations in the two studies, respectively, and heart rates increased significantly in both studies. Noise stimulation resulted in longer murmur durations measured on PCG files as well as increased aortic flow velocities by Doppler echocardiography (paper I). In summary, the higher murmur grades after exercise as well as the longer murmur duration and increased aortic flow velocities after noise stimulation suggest that sympathetic stimulation is an important factor influencing blood flow in the LVOT of boxers with low intensity ejection murmurs.

Progression of systolic ejection murmurs

As can be seen in table 3, the percentage of systolic ejection murmurs in 7 week old puppies (Höglund *et al*., unpublished data) was similar to adult boxers from the other studies. All, but one, of the murmurs detected in the 59 puppies were graded I-II/VI. The remaining murmur was graded III/VI. Low intensity ejection murmurs in young dogs without structural heart disease – often referred to as

innocent or physiologic murmurs - would be expected to diminish or resolve by the age of six months (Kvart & Häggström, 2002; Oyama, *et al.*, 2005; Prosek, 2005), a tendency which was not seen in young boxers enrolled in the investigations included in this thesis. However, based on the study by Pyle *et al*. (1976) in Newfoundlands, it has been suggested that SAS may develop in the post-natal period in boxers. In that case, the murmurs would be expected to become increasingly prominent during the first six months of life (Oyama, *et al.*, 2005). None of the dogs in papers III (experiment 1) or IV developed structural changes consistent with SAS by 2D echocardiography during their first year of life. Nevertheless, the percentage of heart murmurs remained high throughout the study period. At 12 months of age, the age at which dog owners are recommended to get a final evaluation of the cardiac status of their dogs (Bussadori, 2000b; Pyle, 2000), nine out of 16 dogs presented with a murmur observed by cardiac auscultation (paper IV). Using time-frequency and complexity analyses on PCG sound files, no significant differences could be found in murmur characteristics between 7 weeks and 12 months of age (experiment 1, paper III). In the individual dogs, there was a difference in peak aortic flow velocity between examinations of up to 0.7 m/s (paper IV). There was, however, no difference in mean aortic flow velocity between the examinations at 7 weeks and 12 months of age in paper III or between any of the six examinations in paper IV. Hence, although intensities of heart murmurs as well as peak aortic flow velocities varied considerably in individual dogs, no consistent change was perceived for the group as a whole during the growth period.

Aortic flow velocity and systolic ejection murmurs

Normal values for aortic flow velocities in dogs have been investigated by several researchers (Gaber, 1987; Yuill & O'Grady, 1991; Kirberger, Bland-Van Den Berg & Grimbeek, 1992; Sisson & Brown, 1996; Abbott & MacLean, 2003). However, differences in transducer placement and Doppler types (continuous-wave or pulsed-wave) between the studies, as well as the lack of details on heart rates and state of excitement of the dogs, make comparison of the results difficult. Mildly increased aortic flow velocities compared to other dog breeds are common in the boxer breed (Bussadori, Quintavalla & Capelli, 2001; Linde & Koch, 2006), which could be an indication of AS (Kienle, 1998; Bonagura & Lehmkuhl, 1999; Oyama, *et al.*, 2005). Some authors use this criterion alone as a base for the diagnosis (Linde & Koch, 2006), but the diagnosis of aortic stenosis in dogs is controversial (Bonagura, 2001) and there is no consensus among cardiologists with regards to the upper limit of aortic flow velocity. Most normal calm dogs of various breeds have peak aortic velocities less than 1.7 m/s (Kienle, 1998; Bonagura & Lehmkuhl, 1999; Oyama, *et al.*, 2005). In dogs with mildly increased aortic flow velocity (1.7-2.25 m/s) it has been recommended to combine the peak velocity measurement with other echocardiographic findings, such as visual appearance of the anatomic obstruction and detection of disturbed flow as well as auscultation of a systolic ejection murmur, for diagnosis of AS (Bussadori, 2000b; Oyama, *et al.*, 2005). Using these criteria, the survey of 500 Italian boxers revealed a prevalence of SAS of 16%, while the prevalence of heart murmurs was much higher, 53% (Bussadori, Quintavalla & Capelli, 2001). In another study

using aortic flow velocities > 2 m/s as the sole diagnostic criterion for AS, 62% of 55 examined boxers were considered affected by the disease (Linde & Koch, 2006). Interestingly, if dogs with mildly increased flow velocities, but without detectable structural changes in the LVOT were excluded, the prevalence of AS in this study was also 16%, while the prevalence of heart murmurs was 73% after exercise. Since most boxers with low intensity systolic ejection murmurs do not have structural changes consistent with subvalvular aortic stenosis on 2D echocardiography additional reasons, other than SAS, must be sought for the high prevalence of heart murmurs and increased aortic flow velocities in this breed.

The left ventricular outflow tract in boxers

In a recent study by Koplitz *et al*. (2006), indexed LVOT areas were found to be significantly smaller in boxers compared to non-boxer controls, whereas no differences in indexed LVOT areas could be found between boxers with low intensity ejection murmurs and boxers without murmurs. In paper IV, although dogs were grouped depending on whether they had a low intensity murmur or not at 7 weeks of age, all dogs presented with murmurs at some point during their first year of life. No differences were found in size variables during growth and only one of the echocardiographic variables differed between the groups. None of the dogs developed signs of structural changes consistent with SAS by 2D echocardiography. Hence, the overall development of the puppies was similar in both groups. Since all dogs presented with murmurs, it cannot be excluded that all of them had a comparably smaller LVOT than growing dogs of other breeds. In addition to boxers, slightly diminished outflow tract dimensions have also been observed in bull terriers and golden retrievers (Oyama*, et al.*, 2005; Koplitz, Meurs & Bonagura, 2006).

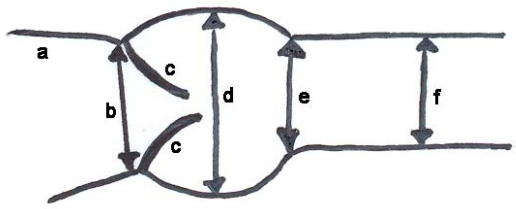

Figure 5. Schematic drawing of the left ventricular outflow tract and the aorta, showing the subvalvular region (a), the aortic valve annulus (b), the aortic valves (c), the Sinus of Valsalva (d), the sinotubular junction (e) and the ascending aorta (f).

In the absence of a subvalvular obstruction, turbulence is most likely to occur at the level of the ventriculoarterial junction where blood passes from a chamber into a long and narrow tube. Hence, the effective orifice diameter (EOD), *i.e.* the smallest diameter of the outflow tract, must be located at the site of, or distal to, the aortic valve annulus (Figure 5). In paper IV, the mean EOD of all 16 dogs was numerically smaller than the subvalvular diameter at all examinations. The differences were, however, very small and not statistically significant. In the study

by Koplitz *et al*. (2006), the indexed diameters at the sinotubular junction as well as in the ascending aorta were significantly larger in non-boxer dogs compared to boxers (both with and without heart murmurs). The investigators found a gradual increase in ejection velocity from the subvalvular LVOT to the ascending aorta, which does not support the presence of a localized stenosis. Studies of 500 Italian boxers have shown smaller indexed diameters of the aortic valve annulus as well as the sinotubular junction compared to other breeds of dogs (Bussadori, 2000a), (Figure 5).

The aorta is an elastic structure with a cyclic change in diameter during the cardiac cycle. One possible explanation for the above mentioned findings is that the end-diastolic diameter of the aorta might be relatively smaller in boxer dogs. Another possibility is a different composition of the aortic wall, with less elastic tissue in boxers, compared to other breeds, a hypothesis that is supported by the low frequency of poststenotic dilatation in boxers with AS compared to dogs of other breeds affected by the disease (Bussadori, 2000a). One or both of these potential causes could result in a smaller systolic diameter. Thus, the high prevalence of low intensity ejection murmurs in the boxer breed could be possibly explained by a smaller size of the LVOT and/or aorta compared to other breeds. Post-mortem examinations, including histopathology, of boxers and other breeds could be useful to answer this question.

One suggested reason for the genesis of innocent murmurs is that puppies have larger stroke volumes for the size of their great arteries compared to adult dogs (Kittleson, 1998). However, in paper IV, LVOT diameters (indexed for body surface area) were larger and when also taking into consideration the higher heart rates in puppies compared to adult dogs, the hypothesis of a larger stroke volume is not supported. Another study showed lower stroke volume and cardiac output as well as higher heart rates, in puppies compared to healthy adult dogs and adult dogs with SAS (Bélanger*, et al.*, 2001). Hence, reasons other than large stroke volumes must be sought to explain the genesis of innocent murmurs in puppies.

Differential diagnoses of AS in boxers include pulmonic stenosis. All dogs included in the investigations of this thesis had normal pulmonic valve leaflets by 2D echocardiography and peak pulmonic flow velocities ≤ 1.6 m/s (Kirberger, Bland-Van Den Berg & Grimbeek, 1992), and as a result pulmonic stenosis could therefore be excluded. In our studies, we have found no evidence of atrial septal defects, reported to be common in a group of adult boxers (Chetboul *et al.*, 2006b).

Aortic insufficiency

Three of the sixteen dogs of paper IV developed AI (aortic insufficiency), detected by colour Doppler echocardiography, before 12 months of age. None of these dogs showed signs of abnormal subvalvular or valvular morphology as seen by 2D echocardiography. AI is a common finding in dogs with subvalvular as well as valvular AS (Kienle, 1998), but is rare in normal dogs (Nakayama *et al.*, 1994) or dogs with other cardiac abnormalities. The only exceptions are ventricular septal defects and bacterial endocarditis of the aortic valve (Kienle, 1998), both of which could be excluded by echocardiographic examination.

The mean value of the left ventricular diastolic diameter at 9 and 12 months of age was significantly larger in the murmur group compared to the non-murmur group, and the three dogs which developed AI all belonged to the murmur group. AI may lead to a larger diastolic left ventricular blood volume, extending the ventricle and resulting in a larger left ventricular diameter. This could then give rise to an increased stroke volume, which in turn could induce heart murmurs and increased flow velocities. However, the variability between examinations in aortic flow velocity was considerable in all sixteen dogs and in the dogs affected by AI there was no direct association between the degree of insufficiency and the aortic flow velocity at each examination. The aortic flow velocity, although slightly increased on a few occasions, was ≤ 2.2 m/s on the majority of the examinations, which is in accordance with the upper limit of 2.25 m/s recommended by Bussadori *et al*. (2000). Thus, the AI was considered clinically irrelevant in these dogs at 12 months of age.

The results in paper I show that 13 of 27 dogs had mild aortic valve thickening on 2D echocardiography. Four of the dogs with aortic valve thickening also had mild AI and two of these also had mild SAS. Dogs with AI (with or without SAS) had significantly higher aortic flow velocity compared to dogs without, whereas neither murmur grade nor aortic flow velocity differed between dogs with or without mild aortic valve thickening.

It is uncertain whether mild valvular changes causing AI in dogs without structural changes in the subvalvular region should be classified as valvular aortic stenosis. Some investigators suggest that AI may be one of the most sensitive indicators of SAS in dogs with the mildest defects (Kienle, 1998), in which the obstruction may not be detectable by 2D echocardiography (Oyama*, et al.*, 2005). However, it is, also possible that valvular changes may occur isolated from subvalvular changes in a higher number than previously estimated. In paper II of this thesis, two of the seven dogs (29%) with AS had valvular stenosis and in paper III, four of eleven dogs (36%) had valvular AS. The relationship between the size of the LVOT, the valvular changes and classic SAS remains to be investigated by genetic testing. However, before this is possible, characterisation of these phenotypes in boxers have to be improved, and until then, breeding counselling will be error prone in the large number of boxers with low intensity ejection murmurs (Oyama*, et al.*, 2005).

Diagnostic techniques in heart murmur evaluation -present and future

Cardiac auscultation

Although the stethoscope has served as an important diagnostic tool in evaluation of cardiovascular disease for many years, the acoustic stethoscope has several disadvantages. It cannot store and play back sounds, nor can it offer a visual display or process the acoustic signal (Tavel, 2006). Additionally the information obtained is subjective and dependent on the expertise of the examiner (Tavel, 1996). With the enormous expansion of other diagnostic techniques, especially ultrasound, cardiac auscultation has received less emphasis in clinical practice as well as in teaching (Mangione *et al.*, 1993). However, with the advent of the

modern, high-quality electronic stethoscope, the limitations associated with the acoustic stethoscope are diminishing. The sound quality is enhanced, sounds can be replayed at full or half speed and the data can be stored for comparison between repeated examinations in the same dog. The sound data can also be transferred between colleagues for consensus or second opinion assessment (Dahl *et al.*, 2002; Tavel, 2006). Additionally some electronic stethoscopes allow simultaneous auscultation by connection of additional earphones to a distributing unit, which is valuable in teaching and research.

In paper I, studies of inter-observer variation in cardiac auscultation of low intensity murmurs in boxers showed the importance of experience of the examiner in performing this examination. In that study, which was the first to be conducted during the work for this thesis, acoustic stethoscopes were used. Whilst acoustic stethoscopes are still the most commonly used, electronic stethoscopes are becoming increasingly wide-spread in small animal practices. In paper IV, in which both types of stethoscopes were used, a slightly higher percentage of heart murmurs were detected using the electronic stethoscope compared to an acoustic one, suggesting that low intensity murmurs might be more easily perceived using an electronic stethoscope.

Analysis of sound signals

The PCG contains complex signals which are difficult to analyze visually. Due to the increasing use of computers and digital signal processing techniques, recent developments such as spectral analysis and time-frequency representations (Durand & Pibarot, 1995) have allowed a revival for PCG as a cardiovascular diagnostic technique. Both time and frequency properties of heart murmurs were investigated in paper III. The selected properties - maximal frequency and duration of murmur frequency >200 Hz - were chosen based on their utility in separating innocent murmurs from those caused by aortic stenosis in humans (Tavel & Katz, 2005). In boxer dogs, it is the differentiation between low intensity murmurs in dogs with or without structural changes consistent with AS by 2D echocardiography which is the most difficult. Analysis of duration of murmur frequency >200 Hz proved useful for separation between these two groups. Maximal murmur frequency did not, however, differentiate between dogs with and without AS, in contrast to previous findings in humans (Tavel & Katz, 2005). In adult humans, aortic stenosis is most commonly an age-related degenerative calcific valvular disease, although discrete subvalvular stenosis does occur (Bonow & Braunwald, 2005). In dogs, aortic stenosis develops at a young age, with the subvalvular form being most commonly reported (O'Grady, *et al.*, 1989; Kienle, 1998; Fox, Sisson & Moise, 1999; Oyama, *et al.*, 2005). The differences in disease characteristics between humans and dogs might affect the murmur properties and thereby the maximal frequency. Another possible explanation could be found in anatomical differences between the species. The human chest is flat, while dogs have narrow deep chests, leading to differences in the position of the heart as well as in the distance from the heart to the thoracic wall. This might lead to variation in dampening of the heart murmurs between humans and dogs. The difference in body size between humans and dogs might also affect the results.

Fractal dimension is a novel property in heart sound evaluation. Through analysis of the waveform of the sound signal, the complexity of the sound can be measured. Variance fractal dimension (VFD) is a technique which operates directly on the waveform of the signal (Kinsner, *et al.*, 2006). However, during our investigations, VFD was found unreliable in the presence of background noise and when the amplitude of the source signal varied between patients with different degrees of stenosis. Hence, VFD proved useful only for analysis of murmurs caused by severe AS, but not for mild stenosis. As a result, the technique had to be abandoned and replaced by the correlation dimension, T_2 (Sprott, 2003). T_2 operates in a reconstructed state space of the signal, which makes the analysis much less sensitive to interpatient variations in the signal to noise ratio. In experiment 2 of paper III, T_2 of the heart murmur was significantly higher in dogs with AS compared to dogs without, indicating a higher complexity of murmurs in dogs affected by the disease. As the stenosis becomes more severe, the blood flow generally becomes more turbulent. With increasing turbulence, the reconstructed state space will depict a more complicated system, thus T_2 will increase. In spite of the low number of dogs with AS (n=11), which prompts caution in interpretation of the results, it was also possible to distinguish between subvalvular (n=7) and valvular (n=4) stenoses by T_2 analysis of the murmurs. The often asymmetric subvalvular stenoses give rise to dynamically changing jets that impinge on one or more of the valve leaflets causing interaction between the flow and several anatomical structures. Hence, from a strictly fluid dynamic point of view, subvalvular AS is likely to cause more complicated flow behaviours compared to valvular AS. Further objective analysis of this finding would require a controlled in vitro setting and would be an interesting future study.

When dividing the dogs of experiment 2, paper III, into subgroups based on aortic flow velocity, dogs with mild AS could not be separated from dogs without structural changes consistent with AS using T_2 alone. By pair-wise combination of the investigated variables, the classification score of the dogs improved. The best separation between dogs with or without structural changes consistent with AS was obtained combining duration of murmur frequency >200 Hz with T_2 giving a sensitivity of 94% and a specificity of 82%.

Paper III comprises the first study using a mathematical model for evaluation of time, frequency and complexity characteristics of heart murmurs in dogs. However, this investigation only included boxer dogs with or without aortic stenosis. Further studies are needed to investigate murmur characteristics in other breeds and other heart diseases. Current TFP and T_2 analyses require advanced mathematical computer systems, which are not available in most animal clinics. Nevertheless, the results of the study provided interesting diagnostic information from heart murmur analyses. With further development of the methods, the analyses could be performed quickly and promptly in a pre-programmed computer, providing a useful diagnostic tool in clinical practice. Time-frequency and complexity analyses could thereby add valuable objectivity to murmur analysis.

Echocardiographic examination

The pressure gradient across the aortic stenosis is used for quantification of disease severity (O'Grady, *et al.*, 1989; Kienle, Thomas & Pion, 1994; Kienle, 1998; Fox, Sisson & Moise, 1999; Oyama, *et al.*, 2005). Cardiac catheterisation, although invasive and expensive, has long been the gold standard for measurements of the pressure gradient in both humans and dogs (Bonagura & Darke, 1995; Davidson, Fishman & Bonow, 2005). In dogs, this procedure requires general anaesthesia, which can cause a reduction in pressure gradient of up to 50% compared to conscious dogs (O'Grady, *et al.*, 1989). This could be one reason why increased pressure gradients were not observed in most dogs even with moderate AS in the study by Pyle *et al.* (1976). Studies in humans and dogs have validated the use of Doppler continuous-wave echocardiography for estimation of the pressure gradient (Currie *et al.*, 1985; Valdes-Cruz, *et al.*, 1985; Smith *et al.*, 1986; Lehmkuhl, *et al.*, 1995). In contrast to cardiac catheterisation, this technique is non-invasive and does not require general anaesthesia or sedation in dogs. Doppler obtained aortic flow velocities are converted to pressure gradients using the modified Bernoulli equation (pressure gradient=4 × maximal velocity^2), (Hatle *et al.*, 1978; Hatle, Angelsen & Tromsdal, 1980).

The recommended subcostal transducer location for aortic flow Doppler examinations (Lehmkuhl & Bonagura, 1994) can cause difficulties in boxers for several reasons. With the transducer placed at the posterior end of the sternum (Figure 3), the distance from the transducer to the point of measurement is around 20 cm in a large boxer. Imaging at a higher tissue depth causes a decrease in resolution (Moise & Fox, 1999), which makes the examination more difficult. In paper II, contrast echocardiography using Optison was investigated in boxers with and without 2D structural changes consistent with AS. In two thirds of the examined dogs Doppler signals before contrast were weak, which caused poor definition of the graph margins and resulted in uncertainty in some of the measurements. Following injection of the contrast agent, a marked enhancement of the Doppler signal was seen in all dogs. The rapid technical advances within the area of diagnostic imaging and the development of newer and future generation ultrasound equipment with improved Doppler capacity will inevitably result in a lower percentage of weak signals. Nevertheless, contrast echocardiography can be an aid in boxer dogs, in which plain Doppler signals are difficult to obtain. In the same study, assessment of myocardial blood flow, a common indication for contrast echocardiography in human cardiac patients with suspected coronary artery disease (Clark & Dittrich, 2000), was investigated. In dogs with moderate-severe AS, this technique could possibly have been useful to identify dogs at risk of myocardial ischemia, but since myocardial opacification was not achieved, assessment of myocardial blood flow was not possible.

Doppler-derived pressure gradients, as well as gradients obtained by cardiac catheterization, are highly flow dependent. If the cardiac output is high, for instance in stressed or excited animals, the pressure gradient may overestimate the severity of an obstruction. In the presence of abnormally low cardiac output (*e.g.* in dogs with concurrent myocardial failure), the pressure gradient will decrease and the severity of an obstruction may be underestimated (Bélanger, *et al.*, 2001;

Oyama, *et al.*, 2005). Therefore, alternative methods for assessment of disease severity have been investigated.

Bélanger *et al*. (2001) used the stroke volume estimated in the right ventricular outflow tract and the aortic velocity time integral to calculate the effective orifice area (EOA) of the LVOT. They found that the EOA indexed for body surface area was a useful and feasible technique for non-invasive assessment of AS severity in dogs, although there was some degree of overlap between dogs with mild SAS and healthy controls. Oyama and Thomas (2002) compared the cross-sectional area at the subvalvular level of the LVOT to the area of the aortic root by 2D echocardiography in dogs. In dogs with moderate and severe SAS, the LVOT/aortic ratio was significantly lower when compared to controls, whereas no difference could be seen between dogs with mild SAS and controls (Oyama & Thomas, 2002). Hence, none of these methods significantly helps in the difficult separation between mild AS and dogs without AS. Likewise, based on the results of troponin measurements in the dogs in our studies as well as in other dogs with AS, this biomarker is not useful for separation between mild AS and non-AS dogs, whereas it could possibly have prognostic value in dogs with severe AS and secondary myocardial injury due to cardiac hypertrophy and ischemia (Oyama & Sisson, 2004). Whether the subtle changes in the LVOT in dogs with mild forms of AS could be discernible by magnetic resonance imaging remains to be investigated.

Until another non-invasive gold standard of diagnosis is identified, Doppler echocardiography remains an important part of the cardiac examination (Bonagura, 2001). However, several factors must be taken into consideration when evaluating the results of the Doppler examination. Since a smaller size of the LVOT has been shown in boxers (Koplitz, Meurs & Bonagura, 2006), it is possible that a slightly higher upper limit of peak aortic flow velocity compared to other breeds should be accepted in boxer dogs (Bussadori, 2000b; Oyama, *et al.*, 2005). Additionally, since stress testing has resulted in significantly higher aortic flow velocities in boxers (Paper I), attention must be paid to the hemodynamic state of the dog at the time of the Doppler recording (Bonagura, 2001; Oyama, *et al.*, 2005). According to investigations of the papers included in this thesis, an upper limit of $\leq$ 2.4 m/s measured by continuous-wave Doppler using the subcostal imaging plane should be considered normal in a boxer dog without structural changes consistent with subvalvular or valvular AS on 2D echocardiography and a heart murmur grade $\leq$ II/VI.

Conclusions

- Experience in cardiac auscultation is important both for the detection and grading of low intensity ejection murmurs in boxers. Excitement caused higher murmur grades, longer murmur duration and increased aortic flow velocities. Therefore, the level of excitement of the dog must be assessed at the time of the examination.

- Contrast echocardiography can be used as an enhancer of subcostal Doppler signals in boxer dogs with and without AS, in which plain Doppler signals are difficult to obtain. Since myocardial opacification was not achieved with the present technique, contrast echocardiography did not allow assessment of myocardial blood flow.

- Using time-frequency analysis, duration of heart murmur frequency > 200 Hz can be used to distinguish murmurs caused by mild AS from murmurs in boxers without structural changes consistent with AS. Combining this analysis with complexity analysis using the correlation dimension (T_2) may be a useful complementary method for diagnostic assessment of cardiovascular function in dogs.

- The variability in both the presence and intensity of low intensity ejection murmurs was high during the first year of life in boxers. Likewise aortic flow velocities varied over time in individual dogs, whereas mean aortic and pulmonic flow velocities in the group of dogs were unchanged. No differences were found between diameters measured at different levels of the LVOT in growing boxers. Aortic insufficiency (AI) can appear at an early age, even though none of the dogs developed SAS. Further studies are needed to evaluate if progression of AI can affect cardiovascular health at an older age.

Clinical implications

- Attention must be paid to the degree of excitement of the dog at the time of cardiac auscultation and Doppler flow measurements, since both the murmur degree and the flow velocity increase in excited dogs.

- In the clinical setting, aortic flow velocities ≤ 2.4 m/s, as measured by subcostal continuous-wave Doppler, in a boxer without structural changes consistent with subvalvular or valvular AS and a heart murmur grade ≤ II/VI should be considered normal.

- Contrast echocardiography can be used as an enhancer of Doppler signals in boxer dogs in which plain Doppler signals are difficult to obtain. However, due to the risk of adverse side reactions after repeated injections, contrast media not containing human serum albumin should preferably be used.

- Experience is important in cardiac auscultation of low intensity murmurs. With further evaluation and development of the techniques, time-frequency and complexity analyses could be useful complements in clinical evaluation of heart murmurs in dogs.

- Whether a boxer puppy has a low intensity ejection murmur at 7-8 weeks of age or not, is of poor prognostic value for the cardiac development of the dog up to the age of 12 months.

- The variability in presence and intensity of low intensity ejection murmurs is high during the first year of life in boxers. The aortic flow velocity can vary by up to 0.7 m/s between examinations in growing boxers without any structural changes consistent with AS being apparent on 2D echocardiography.

- Mild-moderate AI may develop in asymptomatic boxer dogs during the growth period. The clinical relevance of this finding for the dog at an older age remains to be investigated.

Future perspectives

- Post-mortem examinations of the subvalvular area and valve apparatus of boxers without structural obstructions identifiable by 2D echocardiography should be performed, to evaluate the sensitivity of the method.

- Post-mortem examinations, including histopathology, should be performed of the LVOT and aorta in boxers and compared to other breeds of dogs.

- Dynamic MRI, when the technique allows sufficient resolution, could be investigated for examination of the ascending aorta. This part is difficult to reach by echocardiography due to interference of lung tissue. Comparison between differences in aortic size, as well as positioning and angling of the aortic arch between different breeds of dogs could give clues to the origin of systolic heart murmurs in boxers. A disadvantage of this method is, however, the necessity for anaesthesia of the dogs, which affects the circulation and could make subtle changes difficult to identify.

- With increased understanding of the relationship between the size of the LVOT, valvular changes and classic SAS in boxers, better characterisation of the phenotypes could be achieved. This would significantly aid in future search for genotypes associated with disease.

- Further studies on murmur characteristics in different breeds of dogs with various heart diseases, as well as development of the techniques, could prove that time-frequency and complexity analyses are useful tools for heart murmur evaluation in clinical practice.

Swedish summary

Populärvetenskaplig sammanfattning

Turbulent blodflöde ger upphov till blåsljud som kan höras när man lyssnar på hjärtat med stetoskop. Mellan 50 och 80% av vuxna boxerhundar har ett blåsljud, varav de flesta är låggradiga. En del av dessa blåsljud orsakas av en stenos, som är en förträngning i utflödesområdet från vänster kammare ut i aorta (stora kroppspulsådern). Denna hjärtsjukdom kan diagnostiseras vid en ultraljuds-undersökning där stenosen kan påvisas i en 2-dimensionell bild. Med hjälp av Dopplerteknik kan en förhöjd flödeshastighet, som uppstår till följd av förträngningen, uppmätas i aorta. Grava fall av aortastenos kan leda till svimningar eller plötslig död på grund av den ökade belastningen på hjärtmuskeln som uppstår då blodet ska pressas genom den trånga passagen ut i aorta.

Majoriteten av vuxna boxerhundar med låggradiga blåsljud har dock inte någon påvisbar aortastenos och inga andra sjukdomstecken. Orsaken till deras blåsljud är oklar, men en möjlig anledning är att boxerhundar har en mindre storlek på utflödesområdet från vänster kammare jämfört med andra hundraser. Detta kan leda till turbulens och lindrigt förhöjd flödeshastighet. Det är viktigt att kunna urskilja om ett blåsljud orsakas av aortastenos eller inte, dels för att kunna ställa diagnos och ge en prognos för den enskilda hunden, men även för att välja ut lämpliga hundar för avel. Målsättningen med denna avhandling var därför att studera fysiologin och att kliniskt utvärdera blåsljud i relation till utvecklingen av vänster kammares utflödesområde hos boxerhundar med och utan aortastenos.

Valpar och vuxna boxerhundar undersöktes med hjälp av stetoskop, EKG, ultraljud och fonokardiografi (en grafisk registrering av hjärtljud). Dessutom utvärderades kompletterande metoder för att analysera frekvensinnehållet och komplexiteten hos blåsljuden, samt utökad ultraljudsundersökning med hjälp av ett kontrastmedel som injiceras i blodet.

Hjärtundersökning av boxer kan vara svårt av flera anledningar. Boxern är en trubbnosig ras med trånga övre luftvägar som ger störande andningsljud. Detta gör det svårt att särskilja hjärtljuden när man lyssnar på hunden med stetoskop. Boxern är dessutom en aktiv och livlig hund, vilket ytterligare kan försvåra undersökningen. Vi gjorde därför en jämförande studie mellan veterinärer med olika erfarenhetsgrad. Resultaten visade att erfarenheten är viktig för att kunna urskilja och bedöma låggradiga blåsljud hos boxer. En högre grad av upphetsning hos hunden gav högre blåsljudsgrad, längre blåsljudsduration och högre flödeshastighet i aorta. Det är därför viktigt att ta hänsyn till graden av upphetsning vid bedömningen av hjärtstatus hos en boxer.

Upprepade undersökningar av växande boxerhundar visade att lindriga blåsljud varierar i intensitet mellan grad 0 och 2 (på en 6-gradig skala) under första levnadsåret. Hos de flesta andra hundraser växer lindriga blåsljud bort vid cirka 6 månaders ålder, men det skedde inte hos boxerhundarna. Ingen av hundarna utvecklade heller någon aortastenos, vilket var oväntat eftersom Newfoundlandshundar tidigare visats kunna utveckla sjukdomen under sitt första levnadsår. Trots detta var andelen blåsljud ungefär lika hög vid 1 års ålder som vid 7-8 veckor, vilket skulle kunna förklaras av den mindre storleken hos

utflödesområdet hos boxer som visats i tidigare studier på vuxna hundar. Tre av 16 hundar utvecklade ett lindrigt-måttligt läckage genom aortaklaffarna, vilket inte gav upphov till några sjukdomstecken vid 1 års ålder. Ytterligare uppföljning av dessa hundar får utvisa om klaffläckaget har någon betydelse för hundarnas hjärtfunktion på sikt.

Utvärderingen av kompletterande diagnostiska metoder visade att kontrastmedel kan underlätta Dopplermätningen av aortas flödeshastighet hos boxer. Digital analys av blåsljudsinnehållet visade att durationen av blåsljudets frekvens över 200 Hz är användbart för att särskilja huruvida ett lindrigt blåsljud är orsakat av aortastenos eller inte. Denna analys i kombination med analys av blåsljudets komplexitet visade sig effektiv för att skilja mellan hundar med och utan stenos.

Sammanfattningsvis varierade låggradiga blåsljud i intensitet hos hundarna och ingen tydlig trend mot minskad eller ökad blåsljudsgrad kunde ses under första levnadsåret. Hos både unga och vuxna boxerhundar ökade blåsljudsgraden vid upphetsning, vilket kan bero på snabbt blodflöde i ett förhållandevis litet vänster kammarutflöde. Erfarenhet visade sig vara viktig vid bedömning av låggradiga blåsljud med stetoskop. Digital analys av blåsljudsduration och blåsljudets komplexitet kan därför bli ett bra komplement. Ytterligare utveckling av denna teknik kan komma att väsentligt underlätta diagnosen vid klinisk bedömning av blåsljud hos hundar.

References

Abbott, J.A. & MacLean, H.N. 2003. Comparison of Doppler-derived peak aortic velocities obtained from subcostal and apical transducer sites in healthy dogs. *Vet Radiol Ultrasound 44*, 695-8.

Abdulla, R. 2001. The history of the stethoscope. *Pediatr Cardiol 22*, 371-2.

Almeida, A.G., Sargento, L., Gabriel, H.M., Da Costa, J.M., Morias, J., Madeira, F., David, C., Oliveira, J., Da Cunha, J.C., Vagueiro, M.C. 2002. Evaluation of aortic stenosis severity: Role of contrast echocardiography in comparison with conventional echocardiography and cardiac catheterization. *Revista Portuguesa de Cardiologia 21*, 555-572.

Altman, D.G. 1991. Inter-rater agreement. In *Practical statistics for medical research*. Chapman and Hall. London. 403-409 pp.

Anderson, R.H. & Ho, S.Y. 1998. The architecture of the sinus node, the atrioventricular conduction axis, and the internodal atrial myocardium. *J Cardiovasc Electrophysiol 9*, 1233-48.

Bélanger, M.C., Di Fruscia, R., Dumesnil, J.G. & Pibarot, P. 2001. Usefulness of the indexed effective orifice area in the assessment of subaortic stenosis in the dog. *J Vet Intern Med 15*, 430-7.

Bonagura, J.D. 2001. Problems in the canine left ventricular outflow tract. *J Vet Intern Med 15*, 427-9.

Bonagura, J.D. & Darke, P.G.G. 1995. Congenital Heart Disease. In *Textbook of Veterinary Internal Medicine*. 4th ed. Edited by S.J. Ettinger & E.C. Feldman. W.B. Saunders Co. Philadelphia, PA. 892-943 pp.

Bonagura, J.D. & Lehmkuhl, L.B. 1999. Congenital heart disease. In *Textbook of Canine and Feline Cardiology*. 2nd ed. Edited by P.R. Fox, D. Sisson & N.S. Moïse. W.B. Saunders. Philadelphia, PA. 485-494 pp.

Bonagura, J.D. & Luis Fuentes, V. 2000. The cardiovascular system; Echocardiography. In *Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat*. 5th ed. Edited by S.J. Ettinger & E.C. Feldman. W.B. Saunders Co. Philadelphia, PA. 834-873 pp.

Bonow, R.O. & Braunwald, E. 2005. Valvular Heart Disease. In *Braunwald's Heart Disease. A Textbook of Cardiovascular Medicine.* 7th ed. Edited by D.P. Zipes, P. Libby, R.O. Bonow & E. Braunwald. Elsevier, Saunders. Philadelphia, PA. 1553-1632 pp.

Boon, J.A. 1998. *Manual of Veterinary Echocardiography*. Williams & Wilkins. Baltimore. 478 pp.

Borkon, A.M., Jones, M., Bell, J.H. & Pierce, J.E. 1982. Regional myocardial blood flow in left ventricular hypertrophy. An experimental investigation in Newfoundland dogs with congenital subaortic stenosis. *J Thorac Cardiovasc Surg 84*, 876-85.

Boswood, A. 2004. Editorial: the rise and fall of the cardiac biomarker. *J Vet Intern Med 18*, 797-9.

Buchanan, J.W. 1999. Prevalence of cardiac disorders. In *Textbook of canine and feline cardiology*. 2nd ed. Edited by P.R. Fox, D.D. Sisson & N.S. Moise. WB Saunders. Philadelphia, PA. 457-470 pp.

Bussadori, C. 2000a. Echo patterns in boxers with subaortic stenosis. In *Proceedings of the 18th Annual Veterinary Medical Forum, ACVIM*, Edited by D.J. Davenport & P. M.R. Seattle, WA. 86-87 pp.

Bussadori, C., Amberger, C., Le Bobinnec, G., Lombard, C.W. 2000b. Guidelines for the echocardiographic studies of suspected subaortic and pulmonic stenosis. *J Vet Cardiol 2*, 17-24.

Bussadori, C., Quintavalla, C. & Capelli, A. 2001. Prevalence of congenital heart disease in boxers in Italy. *J Vet Cardiol 3*, 7-11.

Chetboul, V., Sampedrano, C.C., Gouni, V., Nicolle, A.P., Pouchelon, J.L. & Tissier, R. 2006a. Ultrasonographic assessment of regional radial and longitudinal systolic function in healthy awake dogs. *J Vet Intern Med 20*, 885-93.

Chetboul, V., Trolle, J.M., Nicolle, A., Carlos Sampedrano, C., Gouni, V., Laforge, H., Benalloul, T., Tissier, R. & Pouchelon, J.L. 2006b. Congenital heart diseases in the boxer dog: A retrospective study of 105 cases (1998-2005). *J Vet Med A Physiol Pathol Clin Med 53*, 346-51.

Clark, L.N. & Dittrich, H.C. 2000. Cardiac imaging using Optison. *Am J Cardiol 86*, 14G-18G.

Currie, P.J., Seward, J.B., Reeder, G.S., Vlietstra, R.E., Bresnahan, D.R., Bresnahan, J.F., Smith, H.C., Hagler, D.J. & Tajik, A.J. 1985. Continuous-wave Doppler echocardiographic assessment of severity of calcific aortic stenosis: a simultaneous Doppler-catheter correlative study in 100 adult patients. *Circulation 71*, 1162-9.

Dahl, L.B., Hasvold, P., Arild, E. & Hasvold, T. 2002. Heart murmurs recorded by a sensor based electronic stethoscope and e-mailed for remote assessment. *Arch Dis Child 87*, 297-301.

Davidson, C.J., Fishman, R.F. & Bonow, R.O. 2005. Cardiac Catheterization. In *Braunwald's Heart disease, a textbook of cardiovascular medicine*. 7th ed. Edited by D.P. Zipes, P. Libby, R.O. Bonow & E. Braunwald. Elsevier, Saunders. Philadelphia, PA. 177-203 pp.

Durand, L.G. & Pibarot, P. 1995. Digital signal processing of the phonocardiogram: review of the most recent advancements. *Crit Rev Biomed Eng 23*, 163-219.

Edler, I. & Hertz, C.H. 2004. The use of ultrasonic reflectoscope for the continuous recording of the movements of heart walls. 1954. *Clin Physiol Funct Imaging 24*, 118-36.

Ettinger, S.J. & Suter, P.F. 1970. *Canine Cardiology*. WB Saunders Company. Philadelphia, PA. 616 pp.

Fisch, C. 2000. Centennial of the string galvanometer and the electrocardiogram. *J Am Coll Cardiol 36*, 1737-45.

Fox, P.R., Sisson, D. & Moïse, N.S. 1999. Congenital heart disease. In *Textbook of canine and feline cardiology*. 2nd ed. Edited by P.R. Fox, D. Sisson & N.S. Moïse. WB Saunders. Philadelphia, PA. 471-535 pp.

Frankel, R.I. 1996. Centennial of Rontgen's discovery of x-rays. *West J Med 164*, 497-501.

Frey, N., Brixius, K., Schwinger, R.H., Benis, T., Karpowski, A., Lorenzen, H.P., Luedde, M., Katus, H.A. & Franz, W.M. 2006. Alterations of tension-dependent ATP utilization in a transgenic rat model of hypertrophic cardiomyopathy. *J Biol Chem 281*, 29575-82.

Gaber, C.E. 1987. Normal pulsed Doppler flow velocities in adult dogs. In *5th Annual Veterinary Medical Forum, American College of Veterinary Internal Medicine* 923 pp.

Gompf, R.E. 1988. The Clinical Approach to Heart Disease. In *Canine and Feline Cardiology*. Edited by P.R. Fox. Churchill Livingstone Inc. New York. 29-42 pp.

Grech, E.D. & Ramsdale, D.R. 1991. Exertional syncope in aortic stenosis: evidence to support inappropriate left ventricular baroreceptor response. *Am Heart J 121*, 603-6.

Guyton, A.C. & Hall, J.E. 2006. *Textbook of Medical Physiology*. 11th ed. Elsevier, Saunders. Philadelphia. 1116 pp.

Hamlin, R.L. 1999. Normal Cardiovascular Physiology. In *Textbook of Canine and Feline Cardiology*. 2nd ed. Edited by P.R. Fox, D. Sisson & N.S. Moïse. WB Saunders. Philadelphia, PA. 25-37 pp.

Hanna, I.R. & Silverman, M.E. 2002. A history of cardiac auscultation and some of its contributors. *Am J Cardiol 90*, 259-67.

Hansson, K., Häggström, J., Kvart, C. & Lord, P. 2002. Left atrial to aortic root indices using two-dimensional and M-mode echocardiography in cavalier King Charles spaniels with and without left atrial enlargement. *Vet Radiol Ultrasound 43*, 568-75.

Harpster, N.K. & Jones, C.L. 1991 Genes and the heart: congenital heart disease. In *Am Acad Vet Cardiol*, Edited by N.K. Harpster & C.L. Jones. 13 pp.

Hatle, L., Angelsen, B.A. & Tromsdal, A. 1980. Non-invasive assessment of aortic stenosis by Doppler ultrasound. *Br Heart J 43*, 284-92.

Hatle, L., Brubakk, A., Tromsdal, A. & Angelsen, B. 1978. Noninvasive assessment of pressure drop in mitral stenosis by Doppler ultrasound. *Br Heart J 40*, 131-40.

Heiene, R., Indrebo, A., Kvart, C., Skaalnes, H.M. & Ulstad, A.K. 2000. Prevalence of murmurs consistent with aortic stenosis among boxer dogs in Norway and Sweden. *Vet Rec 147*, 152-6.

Higgins, C.B., Stark, D., McNamara, M., Lanzer, P., Crooks, L.E. & Kaufman, L. 1984. Multiplane magnetic resonance imaging of the heart and major vessels: studies in normal volunteers. *Am J Roentgenol 142*, 661-7.

Ho, C.Y. & Seidman, C.E. 2006. A contemporary approach to hypertrophic cardiomyopathy. *Circulation 113*, e858-62.

Häggström, J., Hansson, K., Kvart, C., Karlberg, B.E., Vuolteenaho, O. & Olsson, K. 1997. Effects of naturally acquired decompensated mitral valve regurgitation on the renin-angiotensin-aldosterone system and atrial natriuretic peptide concentration in dogs. *Am J Vet Res 58*, 77-82.

Häggström, J., Kvart, C. & Hansson, K. 1995. Heart sounds and murmurs: changes related to severity of chronic valvular disease in the Cavalier King Charles spaniel. *J Vet Intern Med 9*, 75-85.

Johnson, A.M. 1971. Aortic stenosis, sudden death, and the left ventricular baroceptors. *Br Heart J 33*, 1-5.

Jones, C.L. 1989. Inheritable left ventricular outflow obstruction in the golden retriever. In *Annu ACVIM Forum,* 851 pp.

Junqueira, L.C. & Carneiro, J. 2005. *Basic histology*. 11th ed. The McGraw-Hill Companies, Inc., United States of America. 502 pp.

Kantz, H. & Schreiber, T. 2004. Phase space methods. In *Nonlinear time series analysis*. Cambridge University Press. 30-47 pp.

Katz, A.M. 2006. *Physiology of the Heart*. 4th ed. Lippincott, Williams & Wilkins. Philadelphia, PA. 644 pp.

Kienle, R.D. 1998. Aortic stenosis. In *Small Animal Cardiovascular Medicine*. Edited by M.D. Kittleson & R.D. Kienle. Mosby. St. Louis, MO. 260-272 pp.

Kienle, R.D., Thomas, W.P. & Pion, P.D. 1994. The natural clinical history of canine congenital subaortic stenosis. *J Vet Intern Med 8*, 423-31.

Kinsner, W., Cheung, V., Cannons, K., Pear, J. & Martin, T. 2006. Signal classification through multifractal analysis and complex domain neural networks. *IEEE Transactions on Systems, Man and Cybernetics, Part C 36*, 196 - 203.

Kirberger, R.M., Bland-Van Den Berg, P. & Grimbeek, R.J. 1992. Doppler echocardiography in the normal dog: Part II - factors influencing blood flow velocities and a comparison between left and right heart blood flow. *Vet Radiol Ultrasound 33*, 380-386.

Kittleson, M.D. 1998. Signalement, history and physical examination. In *Small Animal Cardiovascular Medicine*. Edited by M.D. Kittleson & R.D. Kienle. Mosby. St. Louis, MO. 36-46 pp.

Kittleson, M.D. & Kienle, R.D. 1998. Normal Clinical Cardiovascular Physiology. In *Small Animal Cardiovascular Medicine*. Edited by M.D. Kittleson & R.D. Kienle. Mosby. St. Louis, MO. 11-35 pp.

Kolliker, A. & Müller, H. 1856. Nockweis der Negativen Schwankung des Muskelstrom am Naturlich sich contrahireiden Muskel. *Verh Phys Med Ges 6*, 528.

Koplitz, S.L., Meurs, K.M. & Bonagura, J.D. 2006. Echocardiographic assessment of the left ventricular outflow tract in the Boxer. *J Vet Intern Med 20*, 904-11.

Koplitz, S.L., Meurs, K.M., Spier, A.W., Bonagura, J.D., Fuentes, V.L. & Wright, N.A. 2003. Aortic ejection velocity in healthy Boxers with soft cardiac murmurs and Boxers without cardiac murmurs: 201 cases (1997-2001). *J Am Vet Med Assoc 222*, 770-4.

Kvart, C., French, A.T., Fuentes, V.L., Häggström, J., McEwan, J.D. & Schober, K.E. 1998. Analysis of murmur intensity, duration and frequency components in dogs with aortic stenosis. *J Small Anim Pract 39*, 318-24.

Kvart, C. & Häggström, J. 2002. *Cardiac Auscultation and Phonocardiography* Uppsala, Sweden. 128 pp.

Leatham, A. 1987. Auscultation and phonocardiography: a personal view of the past 40 years. *Br Heart J 57*, 397-403.

Lehmkuhl, L.B. & Bonagura, J.D. 1994. Comparison of transducer placement sites for Doppler echocardiography in dogs with subaortic stenosis. *Am J Vet Res 55*, 192-8.

Lehmkuhl, L.B., Bonagura, J.D., Jones, D.E. & Stepien, R.L. 1995. Comparison of catheterization and Doppler-derived pressure gradients in a canine model of subaortic stenosis. *J Am Soc Echocardiogr 8*, 611-20.

Linde, A. & Koch, J. 2006. Screening for aortic stenosis in the Boxer: Auscultatory, ECG, blood pressure and Doppler echocardiographic findings. *J Vet Cardiol 8*, 79-86.

Lord, P.F. & Suter, P.F. 1999. Radiology. In *Textbook of Canine and Feline Cardiology*. 2nd ed. Edited by P.R. Fox, D. Sisson & N.S. Moïse. WB Saunders. Philadelphia, PA. 107-129 pp.

Luis Fuentes, V. 1993. *Aortic stenosis in boxers*. 33rd ed. Blackwell Scientific. London. 220-229 pp.

Mangione, S., Nieman, L.Z., Gracely, E. & Kaye, D. 1993. The teaching and practice of cardiac auscultation during internal medicine and cardiology training. A nationwide survey. *Ann Intern Med 119*, 47-54.

Mark, A.L., Abboud, F.M., Schmid, P.G. & Heistad, D.D. 1973. Reflex vascular responses to left ventricular outflow obstruction and activation of ventricular baroreceptors in dogs. *J Clin Invest 52*, 1147-53.

Miller, M.S., Tilley, L.P., Smith, F.W.K. & Fox, P.R. 1999. Electrocardiography. In *Textbook of Canine and Feline Cardiology*. 2nd ed. Edited by P.R. Fox, D. Sisson & N.S. Moïse. WB Saunders. Philadelphia, PA. 67-105 pp.

Moïse, N.S. & Fox, P.R. 1999. Echocardiography and Doppler Imaging. In *Textbook of Canine and Feline Cardiology*. 2nd ed. Edited by P.R. Fox, D. Sisson & N.S. Moïse. WB Saunders. Philadelphia, PA. 130-171 pp.

Nakayama, T., Wakao, Y., Takiguchi, S., Uechi, M., Tanaka, K. & Takahashi, M. 1994. Prevalence of valvular regurgitation in normal beagle dogs detected by color Doppler echocardiography. *J Vet Med Sci 56*, 973-5.

Nigam, V. & Priemer, R. 2005. Accessing heart dynamics to estimate durations of heart sounds. *Physiol Meas 26*, 1005-18.

Noonan, J.A. 2004. A history of pediatric specialties: the development of pediatric cardiology. *Pediatr Res 56*, 298-306.

Nygaard, H., Thuesen, L., Hasenkam, J.M., Pedersen, E.M. & Paulsen, P.K. 1993. Assessing the severity of aortic valve stenosis by spectral analysis of cardiac murmurs (spectral vibrocardiography). Part I: Technical aspects. *J Heart Valve Dis 2*, 454-67.

O'Grady, M.R., Holmberg, D.L., Miller, C.W. & Cockshutt, J.R. 1989. Canine congenital aortic stenosis: A review of the literature and commentary. *Can Vet J 30*, 811-815.

Oyama, M.A. & Sisson, D.D. 2001. Evaluation of canine congenital heart disease using an echocardiographic algorithm. *J Am Anim Hosp Assoc 37*, 519-35.

Oyama, M.A. & Sisson, D.D. 2004. Cardiac troponin-I concentration in dogs with cardiac disease. *J Vet Intern Med 18*, 831-9.

Oyama, M.A., Sisson, D.D. & Solter, P.F. 2007. Prospective screening for occult cardiomyopathy in dogs by measurement of plasma atrial natriuretic peptide, B-type natriuretic peptide, and cardiac troponin-I concentrations. *Am J Vet Res 68*, 42-7.

Oyama, M.A., Sisson, D.D., Thomas, W.P. & Bonagura, J.D. 2005. Congenital heart disease. In *Textbook of veterinary internal medicine*. 6th ed. Edited by S.J. Ettinger & E.C. Feldman. Elsevier Saunders. St. Louis, Missouri. 972-1021 pp.

Oyama, M.A. & Thomas, W.P. 2002. Two-dimensional and M-mode echocardiographic predictors of disease severity in dogs with congenital subaortic stenosis. *J Am Anim Hosp Assoc 38*, 209-15.

Pedersen, H.D., Häggström, J., Falk, T., Mow, T., Olsen, L.H., Iversen, L. & Jensen, A.L. 1999. Auscultation in mild mitral regurgitation in dogs: observer variation, effects of physical maneuvers, and agreement with color Doppler echocardiography and phonocardiography. *J Vet Intern Med 13*, 56-64.

Prosek, R. 2005. Abnormal heart sounds and murmurs. In *Textbook of veterinary internal medicine*. 6th ed. Edited by S.J. Ettinger & E.C. Feldman. Elsevier Saunders. St. Louis, Missouri. 195-200 pp.

Prosek, R., Sisson, D.D., Oyama, M.A., Biondo, A.W. & Solter, P.F. 2004. Plasma endothelin-1 immunoreactivity in normal dogs and dogs with acquired heart disease. *J Vet Intern Med 18*, 840-4.

Pyle, R.L. 2000. Interpreting low-intensity cardiac murmurs in dogs predisposed to subaortic stenosis. *J Am Anim Hosp Assoc 36*, 379-82.

Pyle, R.L., Lowensohn, H.S., Khouri, E.M., Gregg, D.E. & Patterson, D.F. 1973. Left circumflex coronary artery hemodynamics in conscious dogs with congenital subaortic stenosis. *Circ Res 33*, 34-8.

Pyle, R.L., Patterson, D.F. & Chacko, S. 1976. The genetics and pathology of discrete subaortic stenosis in the Newfoundland dog. *Am Heart J 92*, 324-34.

Roguin, A. 2006. Rene Theophile Hyacinthe Laënnec (1781-1826): the man behind the stethoscope. *Clin Med Res 4*, 230-5.

Sisson, D. & Brown, W. 1996. Pulsed wave Doppler blood flow velocities and transvalvar volume flow estimates in adult male hounds. *J Vet Intern Med 10*

Sisson, D. & Ettinger, S.J. 1999. The Physical Examination. In *Textbook of Canine and Feline Cardiology*. 2nd ed. Edited by P.R. Fox, D. Sisson & N.S. Moïse. W.B. Saunders Company. Philadelphia, PA. 46-64 pp.

Smith, L.A., Cowell, S.J., White, A.C., Boon, N.A., Newby, D.E. & Northridge, D.B. 2004. Contrast agent increases Doppler velocities and improves reproducibility of aortic valve area measurements in patients with aortic stenosis. *J Am Soc Echocardiogr 17*, 247-52.

Smith, M.D., Dawson, P.L., Elion, J.L., Wisenbaugh, T., Kwan, O.L., Handshoe, S. & DeMaria, A.N. 1986. Systematic correlation of continuous-wave Doppler and hemodynamic measurements in patients with aortic stenosis. *Am Heart J 111*, 245-52.

Sprott, J.C. 2003. Correlation dimension. In *Chaos and time series analysis*. Oxford Univ. Press. Oxford. 307-311 pp.

Tavel, M.E. 1996. Cardiac auscultation. A glorious past--but does it have a future? *Circulation 93*, 1250-3.

Tavel, M.E. 2006. Cardiac auscultation: a glorious past--and it does have a future! *Circulation 113*, 1255-9.

Tavel, M.E. & Katz, H. 2005. Usefulness of a new sound spectral averaging technique to distinguish an innocent systolic murmur from that of aortic stenosis. *Am J Cardiol 95*, 902-4.

Thomas, W.P., Gaber, C.E., Jacobs, G.J., Kaplan, P.M., Lombard, C.W., Moise, N.S. & Moses, B.L. 1993. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. *J Vet Intern Med 7*, 247-52.

Tiback, B. & Hedhammar, Å. 1977. *Boxer*. Ica bokförlag. Västerås. 128 pp.

Tidholm, A. 1997. Retrospective study of congenital heart defects in 151 dogs. *J Small Anim Pract 38*, 94-8.

Tidholm, A., Häggström, J. & Hansson, K. 2001. Effects of dilated cardiomyopathy on the renin-angiotensin-aldosterone system, atrial natriuretic peptide activity, and thyroid hormone concentrations in dogs. *Am J Vet Res 62*, 961-7.

Valdes-Cruz, L.M., Jones, M., Scagnelli, S., Sahn, D.J., Tomizuka, F.M. & Pierce, J.E. 1985. Prediction of gradients in fibrous subaortic stenosis by continuous wave two-dimensional Doppler echocardiography: animal studies. *J Am Coll Cardiol 5*, 1363-7.

Winterer, J.T., Lehnhardt, S., Schneider, B., Neumann, K., Allmann, K.H., Laubenberger, J. & Langer, M. 1999. MRI of heart morphology. Comparison of nongradient echo sequences with single- and multislice acquisition. *Invest Radiol 34*, 516-22.

Yuill, C.D.M. & O'Grady, M.R. 1991. Doppler-derived velocity of blood flow across the cardiac valves in the normal dog. *Can J Vet Res 55*, 185-192.

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