Diagnostic Imaging of Cardiopulmonary Structures in Normal Dogs and Dogs with Mitral Regurgitation

Kerstin Hansson
Department of Biomedicine and Veterinary Public Health,
Division of Diagnostic Imaging and Clinical Pathology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2004

Acta Universitatis Agriculturae Sueciae
Veterinaria 167

ISSN 1401-6257
ISBN 91-576-6654-7

Tryck: SLU Service/Repro, Uppsala 2004

Abstract

Hansson, K. 2004. *Diagnostic imaging of cardiopulmonary structures in normal dogs and dogs with mitral regurgitation.* Doctor´s dissertation.
ISSN 1401-6257, ISBN 91 576 6654 7

The general aim of this thesis was to evaluate two different imaging techniques used to evaluate the heart of dogs with mitral regurgitation, one for measurements of left atrial size and one for general cardiac size and to compare cardiopulmonary imaging with plasma levels of natriuretic peptides. A more specific aim was to study the variability in objective measurements of left atrial and cardiac size and subjective evaluation of general cardiomegaly, left atrial enlargement and radiologic signs of congestive heart failure among groups of observers with varying experience in thoracic radiology. The experience levels included were; experienced radiologists, experienced small animal clinicians, trainees for a small animal specialist degree and veterinary students. General heart enlargement can be monitored by objective measurements but there is no way to objectively estimate left atrial size on radiographs. An established index method for measurements of left atrial size on M-mode echocardiogram was compared to an index that uses the two-dimensional (2-D) image for dimensional measurements. It was shown that the 2-D index can be used as a good predictor of left atrial size. The 2-D index had a greater sensitivity for left atrial enlargement compared to the M-mode index.

Plasma levels of natriuretic peptides (ANP, NT-proANP and BNP) were studied as endogenous markers for increase in left atrial and ventricular size and left ventricular dysfunction. The plasma levels of the studied peptides did not increase in any significant way until there was radiologic and echocardiographic evidence of cardiac enlargement. However, the peptides were shown to be efficient in separating compensated from decompensated heart failure.

In the objective measurement of heart size the interobserver variation of the vertebral heart size method had a mean variation among the 16 observers of around 1.0 VHS units. No difference in mean VHS values was found among the four groups of observers. The subjective evaluation had a large discrepancy amongst both the observers and between the observers and the gold standard. In general the observers overdiagnosed signs of congestive heart failure and underdiagnosed left atrial and general heart enlargement. Accuracy in the subjective interpretation of general cardiomegaly, left atrial enlargement and radiologic signs of congestive heart failure was not in any conclusive way affected by observer experience. Objective measurements of cardiac size were independent of observer experience but dependent on individual observers´ selection of reference points for measurements.

Keywords: left atrial size, LA/AO index, atrial natriuretic peptide, NT-proANP, BNP, VHS, interobserver variation, ROC, subjective interpretation, observer experience

Author´s adress: Kerstin Hansson, Department of Biomedicine and Veterinary Public Health, Division of Diagnostic Imaging and Clinical Pathology, Swedish University of Agricultural Sciences, Box 7029, SE-750 07 UPPSALA, SWEDEN,
E-mail: Kerstin.Hansson@klra.slu.se

Bilddiagnostik av hjärta och lungor hos normala hundar och hundar med mitralis regurgitation

Svensk sammanfattning

Avhandlingens generella syfte var att utvärdera två olika bilddiagnostiska tekniker vilka används för att bedöma hjärtat på hundar med mitralis regurgitation, en för mätning av vänster förmak och en för bedömning av generell hjärtstorlek samt att jämföra bilddiagnostik av hjärta och lungor med plasmanivåer av natriuretiska peptider. Ett mer specifikt syfte var att studera variabiliteten för objektiva mätningar av vänster förmak och generell hjärtstorlek samt variabiliteten för subjektiva bedömningar av vänster förmak, generell hjärtstorlek och radiologiska tecken på hjärtsvikt bland en grupp bedömare med varierande erfarenhet av thoraxradiologi. Följande erfarenhetsnivåer var inkluderade; erfarna veterinärradiologer, erfarna smådjurskliniker, veterinärer under utbildning till specialister i kattens och hundens sjukdomar samt veterinärstudenter.

Generell hjärtförstoring kan bedömas med hjälp av objektiva mätmetoder men det finns ingen radiologisk metod för att mäta vänster förmak. Detta kan däremot göras med hjälp av ultraljud. En etablerad ultraljudsmetod är att med hjälp av ett index mäta vänster förmak på M-mode. Denna metod jämfördes med en metod där den tvådimensionella (2-D) bilden används för mätningarna. Resultaten visade att 2-D metoden är en bra metod som kan användas för att mäta storleken på vänster förmak samt att den hade en större känslighet för att påvisa en förstoring av förmaket jämfört med den etablerade M-mode metoden.

Plasmanivåer av natriuretiska peptider (ANP, NT-proANP och BNP) studerades som endogena markörer för förstoring av vänster förmak, vänster kammare samt vänster kammardysfunktion. Plasmanivåerna för de studerade peptiderna steg inte påtagligt förens förstoring av vänster förmak och kammare kunde ses med hjälp av röntgen och/eller ultraljud. Peptiderna kunde däremot särskilja väl mellan kompenserad och icke-kompenserad hjärtsvikt.

I de objektiva mätningarna av hjärtstorlek var medelvariationen mellan de 16 bedömarna i storleksordningen 1 kotenhet. Ingen skillnad i medelvärden kunde påvisas mellan de olika bedömargrupperna. De objektiva mätningarna var inte alls påverkade av bedömarnas erfarenhetsnivå utan påverkades av enskilda bedömares val av avvikande mätpunkter. I den subjektiva utvärderingen fanns stora skillnader både mellan bedömarna och mellan bedömarna och studiens gyllene standard. Generellt sätt så överdiagnosticerades radiologiska tecken på hjärtsvikt medan vänster förmaks- och generell hjärtförstoring underdiagnosticerades. Precisionen i de subjektiva bedömningarna var inte på något avgörande sätt påverkat av bedömarnas erfarenhet.

A picture may be worth
a thousand t tests

Cooper and Zangwill, 1989

To my parents

Contents

Appendix

Papers I – IV

The present thesis is based on the following 4 papers, which will be referred to by their Roman numerals:

I. 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. *Veterinary Radiology & Ultrasound* 43, 568-575.

II. Häggström J., Hansson K., Kvart C., Duelund Pedersen H., Vuolteenaho O. & Olsson K. 2000. Relationship between different natriuretic peptides and severity of naturally acquired mitral regurgitation in dogs with chronic myxomatous valve disease. *Journal of Veterinary Cardiology* 2, 7-16.

III. Hansson K., Häggström J., Kvart C. & Lord P. 2003. Variability among observers of vertebral heart size measurements in dogs with normal and enlarged hearts. *Submitted for publication.*

IV. Hansson K., Häggström J., Kvart C. & Lord P. 2004. Variability among observers evaluating heart enlargement and pulmonary edema on thoracic radiographs in normal dogs and dogs with mitral regurgitation. *Manuscript.*

Papers I and II are reproduced by permission of the respective journals concerned.

Abbreviations

ANP	atrial natriuretic peptide
AO	aorta
AUC	area under the curve
BNP	brain natriuretic peptide
Br-Sp	bronchus-spine
CHF	congestive heart failure
CV	coefficient of variation
DipECVDI	diplomate of European College of Veterinary Diagnostic Imaging
DV	dorso-ventral
ECG	electrocardiogram
HE	heart enlargement
LA	left atrium
LAE	left atrial enlargement
LAu	left auricle
LA/AO	left atrial to aortic root ratio
LV	left ventricle
LVEDD	left ventricular end diastolic dimension
LVESD	left ventricular end systolic dimension
M-mode	motion-mode
MR	mitral regurgitation
NT-proANP	N-terminal fragment of pro-atrial natriuretic peptide
NYHA	New York Heart Association
RAAS	renin-angiotensin-aldosteron system
ROC	receiver operating chareteristic
T4	fourth thoracic vertebrae
VD	ventro-dorsal
VHS	Vertebral heart size
2-D	two-dimensional mode

Introduction

A good friend once said that looking at a radiograph is like looking into the fog. For a second you think you see something and then it fades away into the shadows. In a way it is true, as radiographs are built up of shadows of many structures creating many different shades of gray but with knowledge and training they will become meaningful and contain information. For anyone working with medical images it is important to be able to, with as high accuracy as possible, separate normal from abnormal findings. Even if that can be done, different observers might chose to classify the abnormalities differently and they can be more or less certain of their interpretation. Measurement methods that can be used to help in these decisions are also a source of variation. They can be imprecise, not incorporating the most relevant structure or vary due to variations between different animals. Finally, the observer using these measurement methods might be an even larger cause for variability. This thesis is an effort to determine some of these variables as they affect imaging of mitral valve regurgitation and to evaluate two methods of measurement of heart size.

Thoracic radiology

Thoracic radiology is considered to be one of the most important diagnostic tools in the evaluation of dogs with suspected cardiovascular disease. The shape and size of the heart give information on the type and severity of the disease. The appearance of the lungs gives information about the condition of the pulmonary circulation, particularly fluid content (edema). Even though technical factors like the X-ray generator, X-ray tube, exposure settings, film type and developer can be controlled and optimized, they are only a part of what really is the image. The image depends on numerous other factors such as how the X-ray beam is centered, positioning of the animal, size and conformation of the animal and conditional status of the animal, like the amount of subcutaneous and intra-cavitary fat. For thoracic radiology, phase of respiration and to some extent, the cardiac cycle will influence the image.

A minimum of two orthogonal views is included in a routine thoracic examination. Straight positioning of the dog is critical as obliquity can cause changes in the position of the heart and thus changes in shape that can lead to misinterpretation of the cardiac size (Ettinger & Suter, 1970; Perkins, 1979). Because there are subtle differences in cardiac conformation and position when comparing right versus left lateral projections it is important that the same lateral projection is used in all serial radiographic examinations. This is even more important when choosing the ventro-dorsal (VD) or dorso-ventral (DV) projection as the standard view as there is a substantial difference in cardiac appearance between these two views (Ruehl & Thrall, 1981). Films should be taken in full inspiration if possible as the normal cardiothoracic ration has been shown to increase with as much as 17% during expiration which can result in a false diagnosis of cardiomegaly (Silverman & Suter, 1975). Full inspiration is necessary to maximize the air contrast in the lungs. Artifacts associated with films taken during the expiratory phase are decreased visualization of the pulmonary vessels and generalized increase in pulmonary opacity that might mimic pulmonary venous hypertension and edema (Silverman & Suter, 1975). Even if care is taken to expose the film at peak inspiration to achieve adequate filling of air into the lungs, there are situations when this is more or less impossible. The most common problem is that the dog is panting. Another problem is if the dog is substantially over weight with an accumulation of intra- and extra thoracic fat. This increase in soft tissue opacity surrounding and superimposing the lungs will make them look hazy and will decrease the possibility to evaluate fine details in the lungs. The fat will also be space occupying and cause a compression of the lungs which might impair full inspiration. Large abdominal masses can displace the diaphragm cranially and the pressure from the abdominal organs may have a negative influence on normal breathing pattern. Furthermore, the dog might suffer from a primary upper airway or lung disease that prevents proper inspiration. In these situations small changes in lung opacity should be evaluated with consideration of these effects.

Radiologic appearance of the normal lung

In a normal dog, parts of the pulmonary parenchyma can be visualized on a radiograph. The lung interstitium is seen as lacy soft tissue opacities in which pulmonary vessels, bronchi and lymphatics are located. The larger airway walls appear as thin parallel soft tissue lines that taper and branch into the periphery. If seen end-on, a bronchus will have the appearance of a ring-like structure. The pulmonary vessels are tubular soft tissue opacities located around the airways. On a lateral view the arteries are dorsal and the veins are ventral to their corresponding airway. On a VD or DV view the arteries are lateral and the veins medial to the airway. In a normal dog the arteries and veins should be of equal size at a given level with an increasing diameter closer to the heart (Suter & Lord, 1984; Root & Bahr, 2002).

Radiologic evaluation of heart size

Experienced interpreters use their subjective impression to decide whether the heart is enlarged or not. The subjective evaluation takes into account the relationship of how much space the heart takes up in the thorax compared to lung volume and if the film is taken in full inspiration or not, cardiac shape and size, position of the trachea, breed and conformation variations.

A number of methods to estimate heart size have been suggested (Schulze & Nöldner, 1957; Hamlin, 1968; Uhlig & Werner, 1969; Toombs & Ogburn, 1985). On the lateral view the cardiac apex-to-base (cardiac length) dimensions should be approximately 70% of the dorsal to ventral distance of the thoracic cavity (Schelling, 1995). The cranio-caudal dimension or width of the heart has been defined to be between 2.5 (deep-chested breeds) and 3.5 (barrel-chested breeds) intercostal spaces (Buchanan, 1968; Kealy, 1979). The cardiac width on a VD or DV view is usually 60 – 65% of the thoracic width (Hamlin, 1968; Schelling, 1995). Various measurement techniques using cardiothoracic ratios has been published (Schulze & Nöldner, 1957; Hamlin, 1968) but have been criticized (Lord, 1975). The major weakness was the use of only one view, DV (Hamlin, 1968) or left lateral view (Schulze & Nöldner, 1957). Furthermore because of breed-caused variations of conformation substantial overlap occurred between normal and enlarged hearts when the ratios were used for different breeds.

A more precise determination of cardiac size, the vertebral heart size (VHS) system has been formulated (Buchanan & Bücheler, 1995). In the VHS system, the cardiac dimensions in the lateral projection are scaled against the thoracic vertebral column. The long and short axes of the heart are measured by a ruler or by use of a caliper. The ruler/caliper is positioned along the vertebral column beginning at the cranial edge of the 4th thoracic (T4) vertebrae. The length and the width of the heart is recorded as the number of vertebrae caudal to (and including) the T4 and estimated to the nearest 0.1 of a vertebra. The length and the width are then added to obtain a VHS value, which is a number that represents heart size proportionate to the vertebral lengths of the dog. The suggested mean value for dogs in the initial study was 9.7 ± 0.5v (8.5 – 10.5v). Subsequent studies have shown a need for breed specific VHS values as several breeds had mean VHS values approximately 1 VHS unit higher than 9.7 (Lamb, Wikeley & Boswood *et al*., 2001).

Echocardiography

Ultrasound is high frequency sound waves (>20,000 cycles per second) that are transmitted through the tissue as longitudinal waves until they are, either reflected, refracted or absorbed. How much of the sound that is reflected depends on the tissue´s acoustic impedance and the beam´s angle of incidence. Each returning echo generates one bit of data and many bits together form an electronic image that can be displayed on a TV monitor (Curry, Dowdey & Murry, 1990). The information from the reflected sound waves can be displayed in different modes of which motion-mode (M-mode) and two-dimensional mode (2-D) are commonly used in practice. The difference between these two display options are that 2-D shows multiple scan lines and thus an actual image of the tissue or organ that are being examined whereas M-mode displays only one scan line. To be meaningful this single scan line is positioned over a moving structure like the contracting heart or the opening or closing valvular leaflets. From the produced image measurements of chamber size, wall thickness as well as details of valvular motion can be assessed (Curry, Dowdey & Murry, 1990). Another important technique is Doppler echocardiography (pulsed and continuous wave) that uses the change in frequency of an ultrasound beam that occurs when it is reflected from moving blood cells. Displayed graphically it allows noninvasive evaluation of the timing, direction and character of blood flow within the heart. A further development of the pulsed wave Doppler is color-flow Doppler by which color-coded images of blood flow velocities can be superimposed on 2-D or M-mode images (Kienle & Thomas, 2002).

Transthoracic echocardiographic images can be obtained only from regions were the heart contacts an intercostal region, which is called an acoustic window. By use of these different windows, systematic ways to examine the heart has been recommended (Thomas, Gaber & Jacob *et al*., 1993). The examination usually starts with a 2-D examination using the right parasternal window. This location provides possibilities to evaluate cardiac anatomy and spatial relationships in long and short axis planes. Routine M-mode measurements are also performed from this window. To perform Doppler examinations other windows that allow the ultrasound beam to be as parallel as possible to the blood flow are used (Kienle & Thomas, 2002).

Measurements of heart size

The heart size may be determined by radiography, both subjectively and by measurement, but only echocardiography can be used to measure size of individual chambers. The accuracy of those measurements are however uncertain because they are usually related to body size or body surface area and the accuracy of this method is doubtful as normal values have a wide range (Bonagura, O´Grady & Herring, 1985; Boon, Wingfield & Miller, 1983; Lombard, 1984). Reference data for various cardiac chambers suffer from relatively small sample sizes and a lack of data points at the extreme ends of very small and very large dogs (Bonagura, O´Grady & Herring, 1985; Boon, Wingfield & Miller, 1983; Lombard, 1984). There are also breed related differences showing that some breeds will have rather constant cardiac dimensions regardless of the body weight of the individual dog. (Morrison, Moise & Scarlett *et al*., 1992).

To avoid having to take into account the size of the patient an index using an internal structure could be more accurate provided that the index structure used is a constant as possible between patients and do not change as a result of common forms of cardiac disease. In 1974 the first paper using an internal index for left atrial (LA) size in humans was published (Brown, Harrison & Popp, 1974) and in the beginning and middle of the 80´s similar studies was reported for dogs (Boon, Wingfield & Miller, 1983; Lombard, 1984). The aorta (AO) was chosen as an internal reference as it was considered easy to find and not likely to change in size in the majority of cardiac diseases. Linear dimensions for the LA and AO were used to calculate an LA/AO ratio as an index for LA size. All these early reports were using M-mode derived indices and were not guided by the 2-D image. Not having the benefit of actually seeing the structures that were being measured caused an uncertainty of whether it was always the same structures that were being measured and whether they were always measured in the same way. With this possible lack of consistency there was an obvious risk of high inter- and intraobserver variability. Currently, M-mode measurements are guided by the 2-D image and thus positioning of the cursor line along which measurements are being performed can be more accurate.

Myxomatous mitral valve disease

Mitral regurgitation (MR) due to myxomatous mitral valve disease has been extensively studied during the second half of the 20^{th} century (Detweiler & Patterson, 1965; Das & Taschjian, 1965; Detweiler, Luginbühl & Buchanan *et al*., 1968; Darke, 1987; Häggström, Hansson & Kvart *et al*., 1992; Beardow & Buchanan, 1993; Häggström, Hansson & Karlberg *et al*., 1994; Darke, 1995; Pedersen, Koch & Poulsen *et al*., 1995; Pedersen, Lorentzen & Kristensen, 1996; Häggström, Hansson & Karlberg *et al*., 1996; Häggström, Hansson & Kvart *et al*., 1997; Pedersen, Lorentzen & Kristensen, 1999; Häggström, Hansson & Kvart *et al*, 2000; Hansson, Häggström & Kvart *et al*, 2002; Kvart, Häggström & Pedersen *et al*, 2002). It has been found to be the most common acquired cardiac disease in small breed dogs. In studies by, Das & Taschjian (1965) and Detweiler, Luginbühl & Buchanan *et al*., (1968), it was stated that the basic mechanisms of pathogenesis and etiology was unknown or incomplete. The etiology has still not been ascertained but several studies has been published on multiple aspects of pathogenesis the disease (Häggström, Hansson & Karlberg *et al.,* 1994; Pedersen, Koch & Poulsen *et al*., 1995; Häggström, Hansson & Karlberg *et al*., 1996; Häggström, Hansson & Kvart, *et al*., 1997; Pedersen, Lorentzen & Kristensen, 1999; Häggström, Hansson & Kvart *et al*., 2000; Hansson, Häggström & Kvart *et.al*, 2002; Kvart, Häggström & Pedersen *et al*., 2002). Typical clinical findings in an asymptomatic dog with myxomatous mitral valve disease is a middle aged, small breed dog with a systolic heart murmur heard best over the left cardiac apex that increase in intensity with increased disease severity (Fox, Sisson & Moise, 1999).

If significant parts of the stroke volume flows from the left ventricle (LV) into the LA instead of out the AO there is an initial decrease in systemic blood flow. Cardiac and peripheral compensatory mechanisms are activated to increase heart rate and modulate the extra-cellular fluid compartment. The heart responds to the volume overload from the fluid retention and regurgitant volume by dilation and eccentric hypertrophy (volume overload hypertrophy) (Fox, Sisson & Moise, 1999). These changes in chamber size can be documented by various diagnostic imaging techniques. In vivo, lesions on the mitral valves can only be detected by echocardiography and are seen as echogenic, irregular thickenings of the valves, often more pronounced on the septal leaflet. Occasionally a ruptured chordae tendineae is seen fluttering in the regurgitant jet area in the LA.

Besides the hemodynamic changes in MR there is a complex mechanism of neurohormonal changes that take place. These changes occur as a response to decreased cardiac output and includes an increased adrenergic drive and activation of the renin-angiotensin-aldosteron system (RAAS) (Riegger, Liebau & Holzschuh *et al*., 1984; Holmer, Riegger & Notheis *et al*., 1987) As the disease process becomes worse, several of the compensatory mechanisms can have undesirable effects such as vasoconstriction and excessive retention of salt and water (Tan, Jalil & Pick *et al*.,1991). When the heart becomes enlarged, other mechanisms, like the secretion of different natriuretic peptides (atrial-, brain and C-type natriuretic peptides; ANP, BNP and CNP) act as cardioprotectors and suppress renin and aldosterone secretion (Riegger, 1990). As plasma levels of these peptides increase in response to atrial stretch (ANP) (Burnett, Kao & Hu *et al*., 1986; Wei, Heublein & Perrella *et al*., 1993) and ventricular dysfunction and dilation (BNP) (Wei, Heublein & Perrella *et al*., 1993) the evaluation of the size of the LA, LV and whole heart might be of value in determining at what stage of heart enlargement the peptides are activated.

Diagnostic imaging of mitral regurgitation

Diagnostic imaging in the forms of radiography and echocardiography are regarded as essential tools in the evaluation of mitral valve disease in dogs, both to help in the diagnosis, but even more to help determine the onset of heart failure, when the patient needs to be treated. In research, knowledge of heart size and more specifically size of the left heart chambers, which are directly affected by MR, is important. The progression of heart enlargement can be followed by serial thoracic radiographs to evaluate overall heart size and by serial echocardiographic examinations to measure the size of individual chambers. The radiologic appearance of mitral valve disease has previously been described and a classification into four phases has been proposed (Hamlin 1968, Ettinger & Suter, 1970). The classification is based on subjective evaluation of changes in size and shape of the LA and LV combined with changes of the pulmonary vasculature and the lungs. As these changes rely on subjective evaluation and the progression of the diseases is gradual, and might differ among both breeds and individuals, comparison is difficult. For the single animal this is less of a problem if the clinician has access to repeat examinations and the animal can serve as its own reference. In research, there is a need for an objective evaluation of heart size. The VHS method has the potential to fill this need for overall estimation of heart size on radiographs but so far there is no objective way to estimate LA size radiographically.

Estimation of regurgitant volume and left atrial size

As the LA is the receiving chamber of the regurgitant volume, it is of great interest to have methods estimating changes in LA size. Furthermore, one of the key bits of information in studies of MR is to be able to estimate the regurgitant volume. Ultrasound is better suited to fulfill these needs as the technique allows for both anatomical visualization as well as information on blood flow. By use of color-flow Doppler the regurgitant jet can be demonstrated and several methods have been used trying to quantitate the regurgitant volume (Grossman, Giesler & Schmidt *et al*., 1995). Lately the proximal isovelocity surface area method has been evaluated in dogs with chronic myxomatous mitral valve disease and it was shown that regurgitant fraction correlated well with LA size (Kittleson & Brown, 2003). However, the technique is not completely accurate and can be technically demanding, which might limit its use in clinical practice (Utsunomia, Ogawa & Tang et al., 1991; Grossman, Giesler & Schmidt *et al*., 1995). As the regurgitant fraction correlates to LA size it might be easier to monitor progression of MR by measuring changes of atrial size. Several methods have been presented and the most commonly used is the LA to AO root index (LA/AO) (Boon, Wingfield &

Miller, 1983; Lombard, 1984; Risniw & Erb, 2000; Hansson, Häggström & Kvart *et al*., 2002). Both M-mode (Boon, Wingfield & Miller, 1983; Lombard, 1984) and 2-D (Risniw & Erb, 2000; Hansson, Häggström & Kvart *et al*., 2002) methods to measure and calculate an LA/AO index has been published. The 2-D derived method has the potential to be more sensitive to LA enlargement as it measures the LA body rather than the left auricle (LAu).

In practice the ideal would be if progression of MR could be monitored by use of non-invasive, standardized and simple methods as not all clinics have the equipment and training to perform advanced examinations. Therefore it is beneficial if the severity of MR could be evaluated by use of the common and conventional techniques of radiography and basic echocardiography.

Interpretation of diagnostic images

Every interpreter has his or her own habit or method on how to interpret the images. It is recommended that images be interpreted by looking at various radiologic signs. For each sign a list of possible pathologic processes can be listed and ordered from the most to the least probable cause. When using the “sign” technique approaches like evaluating organ by organ or viewing the film from left to right or from center to periphery has been suggested (Suter & Lord, 1984). This systematic way of looking at images can be used not only for radiographs but for any imaging modality. Occasionally the interpreter surrenders to the “Aunt Minnie” technique. The term "Aunt Minnie" describes an imaging finding that is both perfectly obvious and pathognomonic of a disease (Teele & Griscom, 1998). This technique relies on that the interpreter use the memory of previous experience of specific radiologic appearances which will cause great trouble when he or she is presented to “new” diseases or variations of previously seen changes. Furthermore the interpreter is prone to block him- or her self from evaluating the entire film and consider other differential diagnoses. To improve accuracy in the interpretation of images it is beneficial to have more than one person reading the films. A method that has been proven to increase accuracy in evaluating presence or absence of venous hypertension and pulmonary edema in humans is to have three interpreters evaluating the radiographs using the third independent interpreter to resolve disagreements between pairs of reader (Hessel, Herman & Swensson, 1978). This metod reduced false positive statements by 97% relative to the individual reading (Nørgaard, Gjørup & Brems-Dalgaard et al., 1990; Hessel, Herman & Swensson, 1978). Furthermore, it has been shown that reading films of progressive disease in a chronological order compared to reading films as singles or pairs (left and right side) of the same patient, improved sensitivity (van Der Heijde, Boonen & Boers *et al*., 1999).

Observer variability

Subjective interpretations of radiographs are difficult to standardize. There is a human factor involved in all sorts of interpretation and in imaging the interpreter is actively, but not always consciously selecting and rejecting parts of the image for viewing and in the evaluation of the film he/she is usually adding a great deal of previous experience into this process. This process can thus never be totally standardized and variations in the subjective evaluation can never be prevented. However, it is valuable to have knowledge of the variability of the subjective evaluation so that results can be interpreted in the light of these known ranges of variability.

Experience in recognizing and interpreting the radiological signs and pathological changes is generally considered to be of utmost importance for accurate assessment but this assumption has been tested in a very limited number of studies in veterinary medicine (Lamb, Tyler & Boswood *et al*., 2000; Caylor, Zumpano & Evans *et al*., 2001; Fettig Rand & Sato *et al*., 2003). In the study by Lamb, Tyler & Boswood *et al*., (2000) three observers with different level of experience evaluated thoracic radiographs subjectively and objectively to assess the influence of the VHS method on the accuracy of the radiographic diagnosis of cardiac disease. They found a difference in measurement values that was not related to observer experience and that all observers were moderately accurate in diagnosing cardiac disease when using a subjective assessment. The experienced observer (veterinary radiologist) was the most decisive and used the answer alternatives “definitely normal” and “definitely cardiac disease” to a higher extent compared to the inexperienced observers (medicine resident and veterinary nurse). Studies concerning the influence of experience in measurements of the tibial plateau slope on lateral radiographs showed a significant difference between inexperienced and experienced observers (Caylor, Zumpano & Evans *et al*., 2001; Fettig Rand & Sato *et al*., 2003).

In human medicine several studies have been published concerning the value of experience and training in thoracic radiology for diagnostic accuracy. The results vary, one study showing that qualified radiologists were more accurate than radiology residents and nonradiologist physicians (Potchen, Cooper & Sierra, *et al*., 2000), another showing that the level of experience was not likely to influence diagnostic performance (Quekel, Kessels & Goie *et al*., 2001). Additionally, some authors state that there is a considerable inter-individual variation in basic perceptive skills which is unaffected by radiologic training (Rackow, Spitzer & Hendee, 1987; Herman & Hessel, 1975). Studies have shown substantial interobserver variation among observers who were highly experienced in thoracic radiology (Robinson, Wilson & Coral *et al*., 1999) and that average levels of agreement were only slightly better among the experienced radiologists than between radiology residents and experienced radiologists (Nørgaard, Gjørup & Brems-Dalgaard *et al*., 1990).

ROC analysis

The question of whether different types of images had any impact on the diagnostic accuracy was initially studied in the late 1940s when a Board of Roentgenology was appointed to determine which of four types of chest images (35mm film versus various types of prints) had the greatest diagnostic accuracy to diagnose tuberculosis (Birkelo, Chamberlain & Phelps, 1947). The study had two major difficulties; the true positive rate was not known and the large variation in interpretation among observers obscured the differences among the types of images. At that time there were no tools available to deal with the problem. In 1960, Lusted (1960) first described the relationship between false positive and false negative results by using the receiver operating characteristic curve (ROC), which is a plot of true positives against false positives. This can be expressed by plotting the relationship between true positive ratio (sensitivity) and false positive ratio (1-specificity). A value for the area under the curve (AUC) can then be calculated (Centor, 1991). The highest possible AUC value is 1.0 and reflects a perfect observer performance. A value under 0.5 indicates an observer performance that is equal to a random outcome (Vining & Gladish, 1992). The ROC technique can be used to compare accuracy of methods (different types of images for example) using the same observers (readers of the images), or to compare observers' ability using the same images (Metz, 1978; Hanley, 1989; Centor, 1991; Vining & Gladish, 1992). For thoracic radiology in veterinary medicine the method has been used to assess the value of the vertebral heart scale method in diagnosing cardiac disease, (Lamb, Tyler & Boswood *et al*., 2000;) for evaluation of caudal vena cava size in dogs with suspected right-sided congestive heart failure (Lemhkuhl, Bonagura & Biller *et al*., 1997) and to compare various radiographic protocols when screening dogs for pulmonary metastases (Barthez, Hornof & Théon *et al*., 1994).

Background to this thesis

The LA is one of the most important structures to evaluate in dogs with a murmur characteristic for MR. The size of the atrium is proportional to the regurgitant fraction and the severity of the volume overload of the atrium (Kittleson & Brown, 2003). Until the beginning of the 1980s, radiology was the only available method in veterinary practice to determine whether the LA was enlarged or not. When echocardiography was introduced it was natural to compare the agreement between the two techniques (Lombard & Spencer, 1985). The clinical impression was that an enlarged LA, determined by radiography, was measured to be normal on M-mode echocardiography. This was confirmed by Lombard & Spencer (1985) in a study in which half of the dogs with mild LA enlargement, determined by radiology, had values within the normal range on echocardiography. The M-mode technique is limited by that the cursor line along which measurements can be done is fixed at the transducer. It can only be moved across the field of view like a wiper blade. Due to this limitation, measurements of the LA were of the LAu or underestimated measurements of the atrial body. On a 2-D image, measurements can be made of structures in any different direction on the image plane and thus the maximal diameter of the LA can be measured. Despite this advantage, the standard method was to measure the LA size by M-mode comparing it with the diameter of the AO. During our early work on MR in the cavalier King Charles spaniel the 2-D technique seemed to be better related to the radiologic impression of atrial enlargement than the M-mode technique, which encouraged an evaluation of the method.

Repeat examinations of the same animal has been used to monitor changes in heart size and presences of radiologic signs of congestive heart failure (CHF) in several studies on MR in the cavalier King Charles spaniel (Häggström, Hansson & Karlberg *et al*., 1994; Häggström, Hansson & Karlberg *et al*., 1996; Häggström, Hansson & Kvart *et al*., 1997; Häggström, Hansson & Kvart *et al*., 2000; Kvart, Häggström & Pedersen *et al*., 2002). Occasionally the clinical presentation and radiologic appearance did not match. In these situations the possibility to compare radiographs from previous examinations was considered very valuable for making the radiologic interpretation as accurate as possible. If doubtful if the dog needed to be treated with diuretics or not, the dog was scheduled for a re-evaluation. During the course of a multi-center trial (Kvart, Häggström & Pedersen *et al*., 2002) concerning treatment of myxomatous mitral valve disease several discussions on the radiologic interpretation of presence or absence of CHF were held, because occasionally some discrepancies among the investigators were revealed. For consistency it was decided that the same radiologist should interpreted all films.

The current recommendation for dogs with MR due to myxomatous mitral valve disease is to institute treatment only when signs of CHF are present. As the radiologic determination of pulmonary edema is invaluable in determining this point it is desirable that there is a common agreement on the radiologic interpretation. This is important both from the dog´s point of view (to get proper treatment at the right time) and from a clinician´s or researcher´s point of view if results from various studies using radiologic appearance as a ground for classification are to be reliably compared. With these concerns, it became important to examine observer agreement in evaluation of thoracic radiographs in dogs with MR.

Aims of the study

The general aim of this thesis was to evaluate two different imaging techniques for measurements of left atrial and general heart size and to compare cardiopulmonary imaging with plasma levels of natriuretic peptides in normal dogs and dogs with mitral regurgitation. A further aim was to study the variability in objective measurements of cardiac size and subjective evaluation of cardiac size and radiologic signs of congestive heart failure among groups of observers with varying experience in thoracic radiology. The objectives are further outlined in the following specific aims:

- To describe a 2-D method for left atrial size as an LA/AO index and to evaluate its relation to body weight and the effect of breathing and stage of diastole on the ratio.
- To compare the LA/AO-2-D method to the standard LA/AO-M method in normal dogs and dogs with left atrial enlargement.
- To compare left atrial and left ventricular size to the response of endogenous markers (ANP, NT-proANP and BNP) in dogs with compensated and decompensated mitral regurgitation.
- To study the variability of the VHS method for measuring the heart size in dogs with a range of different heart sizes among observers with different levels of experience in thoracic radiology.
- To investigate the variability among readers of varying experience in thoracic radiology in the subjective evaluation of global cardiac size, left atrial size and signs of pulmonary edema in normal dogs and dogs with mitral regurgitation.

Materials and methods

The thesis is based on radiographic and echocardiographic examinations selected from a large number (>500) of examinations collected from 1990 to 1999, which involved several studies on progression and treatment of myxomatous mitral valve disease in cavalier King Charles spaniels (Häggström, Hansson & Karlberg *et al*., 1994; Häggström, Hansson & Karlberg *et al*., 1996; Häggström, Hansson & Kvart *et al*., 1997; Häggström, Hansson & Kvart *et al*., 2000; Hansson, Häggström & Kvart *et al*., 2002; Kvart, Häggström & Pedersen *et al.,* 2002). All echocardiographic and radiographic studies were performed at the Department of Clinical Radiology, Swedish University of Agricultural Sciences.

Study materials

Paper I: 166 dogs (74 males and 92 females), 56 normal and 110 with MR.

Paper II: 103 dogs (50 males and 53 females), 27 normal and 76 with MR.

Papers III and IV: 50 sets of thoracic radiographs (left lateral and VD views) divided into 10 sets with normal cardiopulmonary structures and 40 with varying degrees of cardiomegaly of which 20 also had radiologic signs of CHF.

In all papers (I – IV), the degree of MR was variable and represented a spectrum expected to be encountered in normal clinical practice. Before the radiographic and echocardiographic examination, all dogs had a clinical evaluation, which included physical examination, auscultation of the heart and electrocardiography (ECG) in that order. The body weight for all dogs was recorded before the clinical examination.

Classification of disease severity (paper II, III and IV)

In study II, radiology and echocardiography was used according to the classification of disease severity in a modified New York Heart Association (NYHA) functional classification scheme, Table 1 (Ettinger & Suter, 1970). Cardiomegaly was determined by radiology and echocardiography and pulmonary congestion/edema by radiology. Dogs in Class O were clinically normal without heart murmur. The NYHA classification system takes into account clinical evaluation as well as cardiothoracic imaging to establish how far the disease has progressed.

The radiographs in paper III and IV were categorized into groups with normal, mild, moderate and severe cardiomegaly with or without radiologic signs of CHF (Table 2) entirely by radiologic appearance and this is not a functional classification like the modified NYHA system. The classification used in paper III and IV was not meant to establish another system but a way to categorize the material for these specific studies.

Table 1

Classification of severity of myxomatous mitral valve disease according to a modified New *York Heart Association (NYHA) functional classification scheme.*
Abbreviations; LA = left atrium, LV = left ventricle

	Cardiac murmur	Cardiomegaly LA and LV	Pulmonary venous congestion and edema	Dyspnea
Class I	Yes	No	No	No
Class II	Yes	Yes	No	No
Class III	Yes	Yes	Yes	Slight
Class IV	Yes	Yes	Yes	Moderate/severe

Echocardiography

All echocardiographic examinations (paper I and II) were performed using a right parasternal window. The heart was scanned in long and short axis planes according to standard procedure (Thomas, Gaber & Jacobs *et al*., 1993). Both M-mode and 2-D measurements for AO and LA were obtained from a short-axis plane at the level of the aortic valves. The 2-D image was used to guide the placement of the M-mode cursor line. In M-mode, AO was measured at end-diastole and LA at end-systole (Lombard, 1984). For the 2-D measurements, (AO and LA) the first frame after the aortic excursion was used, which is early diastole. In 2-D, transverse dimensions of AO and LA were measured. For AO, the first caliper was placed at the midpoint of the convex curvature of the wall of the right aortic sinus. The second caliper was positioned at the point where the aortic wall and the non-coronary and the left coronary aortic cusps merge. The LA was measured by extending the line from the same point where the second caliper was positioned to the blood-tissue interface of the LA wall. The LA/AO ratios were calculated as an index for atrial size in M-mode and 2-D. In study II, dimensions of the left ventricle in systole (LVESD) and diastole (LVEDD) were measured in a short axis plane just below the mitral valves (Thomas, Gaber & Jacobs *et al*., 1993). All measurements were performed twice on non-consecutive heart beats and the mean value was used. One of two ultrasound machines available at different times (Aloka SSD 650, ALOKA CO. LTD, Tokyo, Japan and Interspec, Apogee RX 800, Bothell, Wa, USA) were used, equipped with either a 5 (Aloka) or a 7.5 (Interspec) megahertz sector transducer. A lead II ECG was simultaneously recorded and all studies were archieved on video tape and as prints. The same person (KH) performed and measured all echocardiographic examinations.

To evaluate the variation due to breathing (interdiastolic variation), LA/AO-2-D index was calculated once in early diastole during 10 respiratory cycles of 3 of the normal dogs and of 4 dogs with various degrees of LA enlargement, giving a mean of 30 measurements per dog. Variations in LA/AO-2-D during diastole (intradiastolic variation) were calculated in 10 of the normal dogs and in 30 of the dogs with LA enlargement. The index was calculated on each video frame in diastole, beginning at closure of the aortic valve and ending at the frame before the aortic excursion and opening of the aortic valves. This was done during two consecutive heart beats which resulted in a mean of 10 measurements per dog. Both inter- and intradiastolic variations were calculated after the echocardiographic examinations from the video recordings.

Radiography and Radiology

All radiographic examinations (paper II, III and IV) were performed using a Siemens Polydoros 50 S generator (Siemens-Elema AB, Sundbyberg, Sweden) with a standard rotating anode 150 kilovolt X-ray tube. Focal-film distance was 100 centimeter and exposure setting was at 1.25 milliampere-second and 85 to 99 kilovolt depending on the size of the dog. Exposure data were registered and the same exposure was used for repeat examinations. Regular intensifying screens (Fuji FG-8, Fuji Photo Co., Ltd., Tokyo, Japan) and latitude film (Fuji Super HR-L) were used in all examinations. In all dogs, left lateral and VD views were taken. Attempts were made to expose all films at peak inspiration. If needed, several exposures were made in order to achieve films with the lungs as well inflated as possible. In paper II, all examinations were interpreted by the same person (KH). In paper III and IV, 16 different interpreters evaluated the radiographs.

Selection of radiographs (paper III and IV)

The 50 sets of radiographs were selected from 27 normal dogs and 69 dogs with varying degrees of MR. Each dog had been examined between 2 and 17 times, giving a total of 367 sets of thoracic radiographs to choose from. The radiographs had to be of good technical and diagnostic quality. The basis for the selection was to create a test situation such that measurements and interpretation of the films would not be biased by obvious difficulties in finding the anatomical landmarks, by obliquity of the projection or by uncommon variations of thoracic conformation or extreme obesity.

Observers (paper III and IV)

The observers represented 4 levels of training and experience with 4 individuals per level. The levels were: 1) European Diplomates in Veterinary Diagnostic Imaging (DipECVDI), 2) Clinicians with >15 years experience of small animal clinical practice, 3) Clinicians enrolled in a 3 year national (Swedish) training program towards specialization in canine and feline diseases, all in their 2^{nd} or 3^{rd} year of training (trainees), 4) 5^{th} year veterinary students whose small animal teaching was completed after the 4^{th} year.

Gold standard (paper III and IV)

Criteria for slight, moderate and severe left atrial enlargement (LAE) and global heart enlargement (HE) and presence of CHF were set by one of the authors (KH) (Table 2) based on criteria in the literature and criteria used during previous studies of MR in the cavalier King Charles spaniel (Ettinger & Suter, 1970; Suter

& Lord, 1984; Kvart, Häggström & Pedersen *et al*., 2002). Two experienced radiologists (KH and PL) selected 10 sets of normal radiographs from the 27 normal dogs and 40 sets of radiographs that fulfilled the criteria for the various degrees of cardiomegaly and CHF as the "gold standard" for comparison with the determinations of the selected observers. The films were assigned to one of the 5 following groups with 10 sets in each: Normal) normal cardio-pulmonary structures, I) slight LAE and HE, II) moderate LAE and HE without CHF, II+) moderate LAE and HE with radiological signs of CHF, III+) severe LAE and HE with radiological signs of CHF.

Table 2
Radiologic inclusion criteria for slight, moderate and severe cardiomegaly and congestive heart failure (paper III and IV). Abbreviations; I = slight left atrial and slight cardiomegaly, II = moderate left atrial and moderate cardiomegaly, II+ = moderate left atrial, moderate cardiomegaly and congestive heart failure III+ = severe left atrial, severe cardiomegaly and congestive heart failure. VD = ventro-dorsal view

	I	II	II+	III+
Left atrium	Straight caudal border or slight concavity at the level of atrio-ventricular junction Minimal dorsal deviation of left main stem bronchus on lateral view Normal VD view	Straight caudal border Dorsal deviation and slight compression of left main stem bronchus With or without bulging of left auricle on VD view	Straight caudal border Dorsal deviation and slight compression of left main stem bronchus With or without bulging of left auricle on VD view	Obvious dorsal deviation and compression of left main stem bronchus on lateral view and Obvious bulging of left auricle on VD view
Overall cardiac appearance	Increased width of ventricular area with a rounded apex on lateral and VD view	Trachea dorsally displaced but not more than parallel to the spine on lateral view. Increased width of ventricular area with a generally rounded appearance on both lateral and VD view	Trachea dorsally displaced but not more than parallel to the spine on lateral view. Increased width of ventricular area with a generally rounded appearance on both lateral and VD view	Trachea dorsally displaced, towards the spine on lateral view Heart silhouette occupies the majority of the thoracic cavity on both lateral and VD view
Vascular structures and pulmonary parenchyma	Normal	Normal	Venous dilation and/or diffuse non-structured mainly perihilar increased interstitial opacity	Venous dilation and/or non-structured increased interstitial opacity mainly in the caudal lung lobes

Instructions to observers (paper III and IV)

The protocol included both subjective and objective evaluation of the radiographs. The same written and oral information was given to all observers prior to the evaluation of the radiographs. The observers were informed that the material consisted of thoracic radiographs of one breed (cavalier King Charles spaniel) and included normal dogs and dogs with varying degrees of MR. No information on the proportion of normal to diseased dogs was given. Before the evaluation each set of radiographs had been assigned a random number between 1 and 50 and sorted according to number. Each observer evaluated all films in one sequence that took 3-5 hours.

Objective measurements (paper III)

The VHS method (Buchanan & Bücheler, 1995) for measuring heart size was used with the following clarification as to which structure that should be used as the reference point at the heart base (A) and a modification of the short axis measurement (B). All measurements were done on the lateral view.

(A) The cardiac long axis was to be measured, in millimeter (mm), with a ruler from the base of the heart to the apex. The reference point at the base of the heart was to be the ventral border of the largest of the main stem bronchi seen in cross section assumed to represent the end-on projection of the left cranial lobe bronchus before the bifurcation to the cranial and caudal lobe segments. If there were several stem bronchi of equal size, the most cranial was to be used.

(B) The cardiac short axis was to be measured, in mm, perpendicular to the measurement of the length. The caudal measurement point for width was to be halfway between dorsal and ventral border of the caudal vena cava.

The cardiac long and short axes were transformed from mm into whole and tenths of VHS units by the observers. One VHS unit included the length of a vertebral body and the following disc. Observers transferred the measurements of each axis to the vertebra on the lateral view and measured each axis (in vertebrae to the nearest 0.1 vertebrae) from the cranio-ventral margin of T4 caudally. One person (KH) then summed the two VHS measurements (for long and short axis) after the study.

Two additional measurements were included, the distance between the main stem bronchus and the spine (Br-Sp) and the long axis of the T4. The former was an indicator of whether variations in long axis measurements were likely to come from the reference point at the heart base or the cardiac apex and the latter was an indicator of vertebral length in the thoracic spine.

Subjective evaluation (paper IV)

Three questions were asked for each set of radiographs;

1) Is the dog in congestive heart failure?
2) Is the heart generally enlarged (global enlargement)?
3) Is the left atrium enlarged?

For each of the questions above the observers were asked to circle one of the following alternatives;

1) Definitely No
2) Probably No
3) Probably Yes
4) Definitely Yes

If the observer classified the heart or the LA to be enlarged, he/she was asked to grade the enlargement to be slight, moderate or severe. After the initial question concerning presence of radiological signs of CHF and before subjective evaluation of HE and LAE, the observers were asked to make the measurements presented above. Lastly, the observers were asked to write what criteria he/she used to define CHF, HE and LAE.

Statistical analyses

All statistical analyses were performed with a computerized statistical software package (JMP 3.2 (I), JMP 3.02 (II), JMP 4.02 (III, IV) SAS Institute Inc., Cary, North Carolina, USA). Details on the statistical methods used in this thesis are presented in the separate papers.

Results and discussion

Imaging of left atrial size

Radiography

No method has been published on how to objectively measure the LA on radiographs. Evaluation of LA size is based on subjective criteria first published in 1970 (Ettinger & Suter 1970). The enlarged LA is described as causing a bulge in the caudo-dorsal cardiac contour and an elevation of the distal part of trachea towards the spine. When the atrium becomes very enlarged it will displace the left stem bronchus dorsally on the lateral view. On the DV view the LA forces the stem bronchi apart and the auricle may be visible as a convex bulge along the left border of the heart. These signs are familiar to the majority of veterinarians interpreting thoracic radiographs and thus it was not a surprise that all 16 observers in the subjective evaluation of LA size (paper IV) stated that they used a bulge in cardiac contour as diagnostic criteria for LAE. The observers probably felt rather comfortable evaluating LA size as they answered “definitely” normal or enlarged more often than “probably” normal or enlarged. However, this sign was recognized and evaluated very differently among the observers and, in general, there was a high rate (17.2%) of false negative observations in the evaluation of LA size. The majority of the false negative observations was found among the group I (slight LAE and slight HE) radiographs. In this group of radiographs there was a false negative rate of 63.8%. Level of training and experience in interpreting thoracic radiographs did not affect how this sign was recognized as all groups of observers had a similar high rate of false negative observations. More important, there was total disagreement among the observers in the interpretation of this group of radiographs (group I) and not in any single set of films did all 16 observers agree on whether the LA was enlarged or not. In sub-analysis of how the observers were able to separate normals from group I radiographs, the AUC values were 16% lower than when all groups of radiographs were included in the AUC analyses.

Echocardiography

As all groups of observers underestimated LAE, echocardiography might be a better and more reliable technique to estimate slight increase in LA size. The previously used M-mode derived LA/AO index for LA size has however been inferior to the subjective radiologic interpretation except for severely enlarged LA (Lombard & Spencer, 1985). There are both advantages and disadvantages with the conventional M-mode technique. The advantages are that it is an established and well recognized technique and that the measurement points can be easily defined according to an ECG tracing and, therefore, can be exactly timed to various phases in the cardiac cycle. The main disadvantage is that it is limited to recording points along the line of the cursor which must be positioned to record both diameter of the AO and the LA size. The anatomy allows measurements only of the LAu or an underestimated diameter of the LA body rather than the largest diameter of the LA. Another disadvantage is that the fat pad surrounding the AO is included in the measurement and the size of this fat pad can alter the position of the LAu. The clinical impression from multiple echocardiographic examinations of dogs with MR is that the LA body dilates at an earlier state than the LAu. A technique which includes a more representative dimension of the LA in the LA/AO index is likely to be more accurate. This can be achieved by using the 2-D image for measurements.

In paper I, the conventional M-mode derived LA/AO index was compared to our 2-D LA/AO method in both normal dogs and dogs with MR. No difference was found between the two indices in normal dogs (M-mode 1.01 ± 0.13 and 2-D 1.03 ± 0.09). However, in dogs with MR the 2-D index was significantly greater than the M-mode index, with the 2-D index in general having an 11% higher value. The interpretation of this discrepancy is that the 2-D index is more sensitive to LA enlargement. The association between the mean and the difference of 2-D and M-mode derived indices was described by quadratic regression (Fig. 1). This reveals a minor difference between the two indices for LA/AO values in the 1.0 – 1.25 range. Dogs with these values are normal dogs or dogs with mild MR. As the mean LA/AO increases, the difference becomes greater up to a mean LA/AO of approximately 2.0, where the difference is 0.3. The downhill part of the curve, where the difference between the two indices declines, represents dogs with significant LA enlargement. In these dogs the LAu is likely to be enlarged to almost the same extent as the LA body and thus there is less difference between the indices.

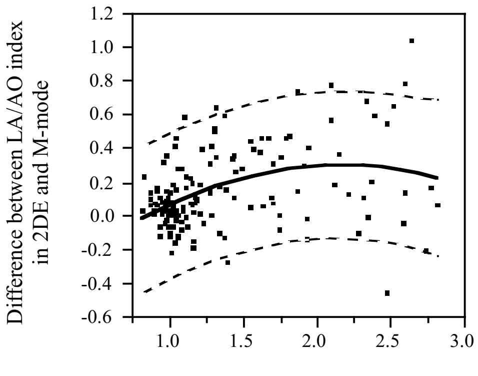

Figure 1. Scatter plot showing the distribution of data for the mean and the difference of 2-D and M-mode derived LA/AO indices. The solid line represents the line of best fit and the dotted line represents 95% prediction interval for individual observations.

As body weight related echocardiographic measurements have been found to have wide confidence intervals, methods that are not related to body size might be more accurate. An index such as LA/AO therefore should be free of any relationship to body weight. In our study (paper I) of both normal dogs and dogs with various degrees of cardiomegaly the LA/AO-2-D index was related to neither body weight nor body weight squared, whereas LA/AO-M was weakly related to both body weight parameters.

It can be difficult to find the correct plane for the measurement due to contraction and rotation of the heart and respiratory movements of the lungs. This difficulty is similar regardless of whether M-mode or 2-D measurements are performed as the 2-D plane used to guide the positioning of the M-mode cursor is the same plane that is being used for the 2-D measurements. A potential problem with the 2-D technique is that measurements are done in early diastole and the LA changes in size and shape during diastole. Another is that the atrium may be different in size at expiration and inspiration due to changes in intrathoracic pressure. In dogs, it has been shown that the LA end diastolic dimension increased significantly with negative pressure but did not change with positive pressure (Katzenberg, Olajos & Morkin *et al.*, 1986). To our knowledge, the effect of breathing on LA size in early diastole has not been published. However, the impact of breathing and stage in diastole on the LA/AO-2D index was found to be low with coefficients of variations (CV) of 3.4 and 3.1%, respectively (paper I).

Obviously there are both advantages and disadvantages with the using an LA/AO-2-D index. The advantages is as stated above that measurement of the LA body is used instead of LAu. The LA body also has a more consistent shape over a larger portion compared to the tapering wedge-shaped LAu. Occasionally a pulmonary vein entering the area where the caliper cross is positioned might cause variations due to the need to extrapolate the border of the LA over the vein.

Relation between LA size and plasma concentration of natriuretic peptides

As the natriuretic peptide ANP is known to be secreted as a response to atrial stretch (Burnett, Kao & Hu *et al.*, 1986) it is of interest to relate plasma levels of this peptide to various degrees of LA enlargement. ANP is secreted in equimolar concentrations to a 98-amino acid N-terminal fragment of proANP (NT-proANP) (Vuolteenaho, Arjamaa, & Ling, 1985). Even though the biological activity of this metabolite is uncertain it is still of interest as a possible biological marker for LA enlargement. In situations of doubt if the LA is enlarged or not, other diagnostic tools like endogenous markers might be of benefit. In this study (paper II) there was however no difference in the plasma levels of the peptides (ANP and NT-proANP) between normal dogs (NYHA, Class 0) and dogs with murmur but no radiographic or echocardiographic evidence of cardiomegaly (NYHA, Class I) (paper II). The reason why plasma levels were not elevated can be either that the MR was minor and thus the LA was almost normal in size or that it takes a larger increase of LA size to activate the secretion of ANP and NT-proANP to a level that is significantly higher than in normal dogs. ANP and NT-proANP levels were increased only with radiologic and echocardiographic evidence of cardiomegaly (NYHA, Class II) but plasma levels were not obviously increased until dogs showed signs of decompensation (NYHA, Class III and IV). In dogs with radiologic signs of pulmonary congestion (venous hypertension) and edema but only slight dyspnea (NYHA, Class III) there was a threefold increase and a sevenfold increase in dogs with severe dyspnea and edema (NYHA, Class IV) was found compared to plasma levels in normal dogs. These results shows that the natriuretic peptides (ANP and NT-proANP) cannot reveal LA enlargement at an earlier state then radiology or echocardiography.

The increase in plasma concentration of BNP was less compared to the increase of the plasma levels for ANP and NT-proANP. It was not until the dogs had cardiomegaly and clinical signs of slight dyspnea (NYHA, Class III) that BNP levels increased and was then approximately twice as high as in Class 0, I and II dogs. The increase in plasma levels of BNP was correlated to LVEDD and to a lesser extent to LVESD. As a conclusion, natriuretic peptides are more efficient in separating decompensated heart failure from compensated than in separating normal heart size from cardiomegaly in dogs with MR.

Observer variability in measuring and interpreting radiographs

Measurements of heart size by use of the VHS method (paper III)

The VHS method by Buchanan (Buchanan & Bücheler, 1995) was developed with the goals to design a method for measurements of cardiac size that would be easy to explain and use, reasonably precise and with justifiable anatomical landmarks.
The method has been used in several studies (Buchanan & Bücheler, 1995; Melián, Stefanacci & Peterson *et al.*, 1999; Litster & Buchanan, 2000a; Litster & Buchanan, 2000b; Lamb, Tyler & Boswood *et al.*, 2000; Lamb, Wikeley & Boswood *et al.*, 2001, Nakayama, Nakayama & Hamlin 2001, Sleeper & Buchanan, 2001) with varying conclusions on the usefulness of the method. In a

study by Lamb, Tyler & Boswood *et al*., (2000) it was concluded that little was to be gained by measuring VHS when evaluating radiographs of dogs with suspected cardiac disease. However, this study included dogs (18 of 50 cases) with cardiac diseases that do not necessarily result in cardiomegaly such as aortic stenosis, dysrythmias and chemodectoma so the material created a bias against the method. In dogs with hypoadrenocorticism significantly lower VHS values was found when compared to normal dogs and the method was found to be a useful criteria in evaluating the suspicion of a smaller than normal heart (Melián, Stefanacci & Peterson *et al*., 1999).

As the presence of MR is usually detected by auscultation, the detection of small increase in heart size, whether by subjective or objective methods, is of lesser importance than the determination of heart size when the cause of dyspnea or lung infiltrates is unclear. In these cases, VHS might be more accurate than a subjective determination. Buchanan & Bücheler (1995) stated that one of the main merits of VHS could be the possibility to follow changes in heart size in the same dog with a precise and objective index. The variability of the method has been tested to a limited extent. Studies on intra-observer variability has not been published and the studies on inter-observer variability involves either few interpreted images (Nakayama, Nakayama & Hamlin, 2001) or few interpreters (Lamb, Tyler & Boswood *et al*., 2000). Nakayama, Nakayama & Hamlin (2001) found a high reproducibility with low CV values when 9 trained observers measured VHS, but the study included only two dogs, one normal (CV 2.7%) and one with severe cardiomegaly (CV 2.8%).

In paper III we found similar low CV values (1.5 – 3.2%) with minimal variation (0.1 – 0.2) in mean VHS values among the 4 groups of observers despite very different experience in thoracic radiology. However, the mean difference among the 16 observers when comparing VHS values for each of the 50 sets of radiographs was approximate 1.0 VHS (1.05 ± 0.32) units regardless of the size of the heart. This is somewhat higher than reported by Lamb, Tyler & Boswood *et al*., (2000) who found a maximum likely difference (mean difference ± 1.96 SD) of >1.0v among the three observers performing the measurements. The higher difference in our material is probably a reflection of that we included 16 different observers compared to 3 observers in the study by Lamb, Tyler & Boswood *et al*., (2000). This rather high mean difference among observers shows that it is crucial to have a common agreement on how measurements should be performed amongst clinicians monitoring the same dog. Even though there is no data showing that intraobserver variation is lower than interobserver variation, it is probably preferred that the same clinician measures all follow up radiographs in a specific case. Furthermore, the high mean difference indicates that normal values for VHS should be used only as guidelines separate normal from enlarged hearts. Another reason why the VHS value should be used as a guide rather than absolute cut-off values is that differences do exist among various breeds. In the original study, 9.7 ± 0.5 was the recommended value for normal heart size but the study included a number of dogs of different breeds. It was suggested by Buchanan that further studies was required to determine more precise values for indivdual breeds. Subsequent, breed specific studies have found a spectrum of normal values from

9.7 ± 0.5 (German shepherd and Yorkshire terrier) to 11.6 ± 0.8 (Boxer) (Lamb, Wikeley & Boswood *et al*., 2001). The cavalier King Charles spaniel has been found to have a normal VHS value of 10.6 ± 0.6 (Lamb, Wikeley & Boswood *et al*., 2001) and we found a very similar value 10.8 ± 0.5.

Causes of variation in individual VHS values

The measurement of cardiac long axis varied more than measurements of cardiac short axis in our study. The mean difference among the observers was around 13 mm for long axis and 6 mm for short axis. According to Nakayama, Nakayama & Hamlin (2001) it is commonly thought that determination of the ventral aspect of the left main stem bronchus can be done relatively easily and in a reproducible manner and that the variations in determining cardiac long axis are due to difficulties in determining the point of the apex. The experience from our study (paper III) is that individual observers can differ substantially from the others. In our study, one observer consistently measured a shorter cardiac long axis, and thus a lower VHS value, than the other observers. This observer measured a longer distance between the left main stem bronchus and the spine (Br-Sp), indicating that the observer had difficulty in defining the reference point at the heart base. In our experience it is sometimes difficult to know which bronchus is which at the heart base. If the dog is slightly rotated the relative position of the stem bronchi is changed. In some dogs several bronchi of equal size are seen end-on in the heart base area. For this reason we decided to clarify more precisely what bronchus that was to be used by the observers in our study (paper III). The observers were instructed to use the largest bronchus that is seen end-on as this is assumed to be the bronchus to the left cranial lung lobe before the bifurcation to the cranial and caudal segment. We are aware of that when several bronchi of equal size are seen the most cranial can be either the right cranial lobe bronchus or the bronchus to left cranial segment (Zontine, 1975). However, the minor distance between the bronchus to the left cranial lung lobe and these two bronchi is not likely to be responsible for the discrepancies in the measurements of cardiac long axis in our study.

In another and later study by Buchanan (2000) the cardiac long axis in dogs with LAE was measured from a reference point caudal to that in the original study to be sure to include the LA. We did not instruct the observers in our study to use this reference point, as this would have introduced an additional source of variation. As the VHS value is related to the vertebrae, variations in vertebral length and presence of narrow intervertebral discs are further sources of variation that should be kept in mind, specially if the subjective impression of cardiac size differs substantially from the expected VHS value.

Subjective evaluation of CHF (paper IV)

The interpretation of radiographs is often described as an art rather than a science. For example, if a pulmonary lesion is subtle, one interpreter might call it a suspicious area whereas another interpreter might dismiss the lesion as a confluence of shadows. In our study, the observers were often suspicious of a lesion, in this case, presence of an increased opacity (edema) in the caudo-dorsal lungfield as criterion of CHF. All observers except for one of the trainees who underdiagnosed CHF, overdiagnosed radiologic signs of CHF. In the 30 sets of radiographs that according to the gold standard had no signs of CHF, the false positive frequency was 18%. The most striking result in general was the discrepancy both among the groups of observers and between the observers and gold standard in the interpretation of individual sets of radiographs. In only one third (17 of 50) of the sets of radiographs did the 16 observers agree with each other and with the gold standard on absence or presence (definitely or probably) of radiological signs of CHF.

Whether early signs of pulmonary edema can be readily detected on radiographs or not has been extensively studied. In the report from a symposium on pulmonary edema (Pistolesi, Miniati & Milne *et al*., 1985) the authors concluded that in humans, chest radiographs could be used, and this method was the only practical mean for early detection and quantification of interstitial edema. In contrast, in a study by Snashall, Keyes & Morgan *et al*., (1981) concerning dogs, the authors came to the conclusion that pulmonary edema could be reliably diagnosed only when extravascular lung water (edema) was increased by 35 percent. The discrepancy between these two reports is suggested to be due to species differences in the lung tissue with poor development of interlobar septa in the dog (Staub, 1974).

Pulmonary edema is seen as a diffuse interstitial or alveolar opacity in the lungs. The pattern seen is a reflection of disease severity, the interstitial pattern preceding the alveolar pattern in the common development of pulmonary edema. (Ettinger & Suter, 1970; Suter & Lord, 1984). Of the two patterns for increased lung opacity the interstitial pattern is the most challenging, because it is both more difficult to detect and because a large number of different causes most be considered. These include nonpathologic factors such as underexposed radiographs, underinflated lungs due to the film being exposed during expiration or increased opacity caused by obesity. The list of pathologic changes that can cause an increased interstitial opacity in the lungs is extensive. However, in our study the interpreters were informed that the only disease involved was mitral regurgitation due to myxomatous mitral valve disease. Furthermore, we included only films with good technical and diagnostic quality. It is speculative to point out the causes for the high rate of false positive observations in our material but one cause might be that the enlarged heart occupies space in the thoracic cavity and compresses the lungs. Compression of the lungs will cause an increase in the opacity that might mimic pulmonary edema. A relatively smaller and more radio-opaque area of the lung is visualized and this is more difficult to evaluate. However, this does not explain the 27 observations (by 10 different observers) of probably or definitely signs of CHF in sets of normal radiographs and sets with slight HE and LAE. Most likely, there are numerous factors involved and the causes for the overdiagnosing cannot easily be identified.

One of the aims of this thesis was to evaluate whether experience in interpreting thoracic radiographs increased accuracy. The results are not unequivocal for all variables that were studied. For interpretation of CHF, 3 of the 4 most experienced interpreters (DipECVDI) had high AUC values both in the evaluation of all films and in the evaluation of films that was expected to be most difficult to interpret (moderate cardiomegaly with or without CHF). When the observations were dichotomized into two alternative by adding the "Yes" alternatives (definitely and probably Yes) on one side and the "No" alternatives (definitely and probably No) on the other, these 3 interpreters agreed with the gold standard for all sets of films with moderate cardiomegaly but without signs of CHF and in 6 of the 10 sets with moderate cardiomegaly and signs of CHF. However, for the entire material (all 50 sets of films) the students were the most accurate group and agreed with the gold standard in 37 sets of films compared to 29 for the DipECVDI. The experienced clinicians had the second highest mean AUC values but agreed with the gold standard in only half of the sets of radiographs when observations was dichotomized. The results are equivocal concerning the effect of training for all aspects of the study. The result from this study clearly shows that the observers evaluated presence or absence of increased opacity as a sign of CHF very differently as they all stated that they used this criterion. In the clinical situation it is likely that some of the technical as well as the animal related difficulties we avoided occur and films of lesser quality are accepted. It is likely that the variability and errors in the interpretation of radiographs would be even more pronounced.

General heart enlargement

The presence of cardiomegaly can provide a clue to whether increased lung opacity is due to cardiogenic pulmonary edema or not. However, cardiomegaly by itself may cause a bias in the evaluation, as it is more probable that a dog with obvious cardiomegaly has pulmonary edema rather than other lung diseases. In this study (paper IV) the observers were probably biased by the presence of increased pulmonary opacity in the evaluation of whether the heart was enlarged or not. This was evident when response data from the two groups of radiographs with moderate cardiomegaly (with or without CHF) was compared. In radiographs with signs of CHF, the observers answered, "definitely enlarged" to a higher extent compared to in sets of films with moderate cardiomegaly but without signs of CHF.

In general, cardiomegaly was underdiagnosed with a false negative observation rate of 28.9%. The majority of the false negative observations were done in the group of radiographs with slight cardiomegaly in which a false negative observation rate of 74.4% was found. Not in any of these sets of radiographs did the observers agree with the gold standard. The observers used a more diverse list of criteria in their evaluation of HE than in the evaluation of LAE and CHF. They were also less precise in the description of the criteria they used. It seems that the question on whether the heart was enlarged or not was the question that was most difficult for the observers to answer among the three questions in the subjective evaluation study (paper IV). It is also likely that criteria for evaluation of heart size differs among various individuals.

Combining the subjective evaluation with measurements of the heart by the VHS method might be a way to increase accuracy. However, it is not likely that a distinct differentiation between a normal heart and a slightly enlarged heart will ever be possible to make with great accuracy unless serial examinations are done and films of a dog with suspected cardiomegaly can be compared with base-line films taken when the dog was truly normal. Even under these circumstances, it is likely that there will an overlap between true/false negative and true/false positive evaluations of cardiac enlargement. The clinical consequence of not being able to distinguish a normal heart from a slightly enlarged heart is minor. However, in the research situation is often needed in order to accurately classify dogs in studies of progressive cardiac disease that cause enlargement of the heart. Possibly this can be achieved by the same observer measuring the heart by the use of the VHS method. However, intra-observer variations need to be quantitated in further studies.

Observer confidence

In the subjective evaluation of presence or absences of CHF, HE and LAE the observers were asked how confident they were in their evaluation by choosing one of the alternatives “definitely” or “probably” “Yes” or “No”. There was a distinct difference between the groups of experienced observers (DipECVDI and experienced clinicians) and the students, the latter being the group with the least experienced observers. The students used the answer alternative “probably” to a higher extent than the experienced interpreters both in the evaluation of CHF and HE. This decision is likely to reflect the fact that the experienced observers are more used to having to make “Yes” or “No” decisions in their daily work whereas the students with limited experience of making decisions affecting patient care were more hesitant to commit themselves.

Comments on the design of a subjective test (paper IV)

In diagnostic tests that involve a subjective evaluation (such as interpretation of radiographs) some sort of confidence threshold is established in the mind of the interpreter. If an image suggests the possibility of disease – how strong must that suspicion be in order for the finding to be classified as positive (*i.e.* signs of disease)? The confidence threshold that an observer adopts undoubtedly depends on many things, his/her “style”, estimation of prior odds or probabilities, assessment of consequences of various possible correct and incorrect decisions. This variation is desirable in a test situation as it mimics the clinical situation. Variations in decision threshold from decision to decision degrade the performance and will have an effect on the ROC curve. This effect is desirable as any aspect of decision making behavior that degrades decision performance should be included in an analysis of an observer-test combination (Metz, 1978).

When evaluating a diagnostic test that relies on subjective evaluation, detectability of the signs interpreted must be taken into consideration. The results from the test can be poor either if the image contains little information or if the observer is not skilled in interpreting the detected information or both (Metz, 1978). The radiographs in our study that according to the authors´ gold standard were classified as slight HE and slight LAE were the films that caused the highest rate of false negative observations, 74.4 and 63.8% respectively. An explanation for this underdiagnosis is that the radiologic signs are less obvious than on films with moderate and severe HE and LAE.

Mathematically it is of equal importance to distinguish true positives from false positives as true negatives from false negatives in a test situation, and it is thus customary to attempt to use approximately equal numbers of actually positive and negative cases in an ROC experiment (Metz 1978). In the clinical situation it is often more important to detect true positive observations *i.e.* signs of disease. In our studies the 50/50 recommendation was roughly achieved for the subjective interpretation of CHF but not for evaluation of general cardiomegaly and LA size. To fulfill this recommendation either the number of radiographs with cardiomegaly should have been decreased to include only 10 sets (as there were 10 sets of normals) instead of 40 or an additional 30 sets of normal radiographs should have been added. The latter would have caused an imbalance in the proportion of sets of films with presence or absence of CHF. The ideal might have been to have two different test situations; one with the question of cardiomegaly or not and one with the question of CHF or not. For practical reasons the present design of the test was chosen.

A further concern in the design of a study involving interpretation of radiographs is viewing time. It has been shown that viewing time affects the accuracy of detection of lesions. True positive observations are clustered in the early viewing period, whereas prolonged viewing seems to increase the number of false positives (Christensen, Murry & Holland *et al.*, 1981). There was no such pattern in our study as the observers using the longest viewing time (5 hours) did not perform differently from the observers using a short viewing time (3 hours) in the evaluation of the last radiographs in the sequence. Furthermore, a high number of sets of films caused disagreement both among observers and with the gold standard. These sets of films were evaluated in the beginning, middle and end of the sequence, not at any particular time.

Future perspectives

Many questions concerning imaging of mitral regurgitation was risen during the work with this thesis. Dogs with mitral valve regurgitation and murmur without apparent clinical signs are often a dilemma for the veterinarian who is asked by the owner to make a prognosis. The actual size of the heart could be a prognostic indicator, but no data have been published on the progression of heart enlargement over time. It would be of interest to study disease progression from an asymptomatic stage to a stage of decompensated heart failure by use of simple measurement techniques like LA/AO-2-D and VHS. Nakayama, Nakayama & Hamlin (2001) found that LA/AO-2-D index measured on a long axis plane were correlated to VHS in a rapid ventricular pacing model for cardiomegaly. It would be of interest to study if there is a correlation between the 2-D index described in this thesis and VHS in naturally occurring MR.

Another interesting perspective would be to investigate the value of measuring plasma levels of ANP or NT-proANP together with measurements of LA/AO-2-D and VHS and to evaluate if any of these methods can be used as prognostic indicators of CHF within a certain time period.

Further studies of VHS and the LA/AO-2-D method in other breeds are still warranted as well as studies on intra-observer variations both for the LA/AO-2-D and the VHS method.

Reading films in chronological order of patients with progressive disease has been proven to improve sensitivity on human patients (van Der Heijde, Boonen & Boers *et al*., 1999). In our study on subjective evaluation of thoracic radiographs the clinically most relevant observer difference was the discrepancy among the observer in evaluating presences or absence of radiologic signs of CHF. An extension of the present study would be to evaluate if the observers would interpret the films differently if they had access to serial examinations of the dogs .

Conclusions

- The LA/AO-2-D index can be used as a good predictor left atrial size. It is independent of body weight and can be measured with good accuracy regardless at what stage of respiration the measurement is done. There are small differences in index values if measurements are made at different times during early diastolic emptying of the left atrium.

- The 2-D index has a greater sensitivity for left atrial enlargement than the M-mode index as it includes dimensions of the atrial body rather than the left auricle.

- The atrial natriuretic peptides (ANP and NT-proANP) can be used as endogenous markers of left atrial enlargement but not at an earlier stage than radiography or echocardiography.

- BNP does not appear to be a good diagnostic marker for early and progressive left ventricular dilation.

- The atrial natriuretic peptides (ANP and NT-proANP) are good indicators of congestive heart failure and can thus be of value in situations of discriminating whether a dog with clinical signs of dyspnea and radiologic signs of increased lung opacity has primary lung disease or congestive heart failure.

- The mean difference among sixteen observers with varying experience in interpreting thoracic radiographs when measuring cardiac size by use of the VHS method was around 1.0 VHS units. Measurements of cardiac long axis varied more than measurements of cardiac short axis.

- Measurements of cardiac size by use of the VHS method does not vary with the experience the observer has in interpreting thoracic radiographs. It varies with individual observers selection of what reference point that is used for the measurement.

- In subjective evaluation of cardiomegaly and radiologic signs of congestive heart failure there was a large discrepancy amongst both the sixteen observers and between the observers and the gold standard set by two experienced radiologists.

- There was no conclusive evidence that experience and training in thoracic radiology improved accuracy in interpretation of presence or absence of cardiomegaly and radiologic signs of congestive heart failure. In general there was an overdiagnosis of congestive heart failure and an underdiagnosis of general cardiomegaly and left atrial enlargement

Acknowledgements

The studies were carried out at the former Department of Clinical Radiology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences (SLU) presently the Department of Biomedicine and Veterinary Public Health, Division of Diagnostic Imaging and Clinical Pathology.

During the period it has taken to complete this thesis, many people have been involved at various times. I would like to express my sincere gratitude to all of you who have directly or indirectly contributed to this work, and especially to the following;

Professor Jens Häggström, my main supervisor, for being able to be both a friend and a supervisor. For always being enthusiastic and present whenever I needed help and support. Reading all my various attempts to write a scientific paper almost always within 24 hours must be some sort of record. Thanks for putting up with my “last minute” way of working and my reluctance to change my manuscripts – you were often right at the end. Congratulations to your recent promotion – it is very well earned !!

Professor Clarence Kvart, my co-supervisor, for being “the hub” in all our studies. All the hours you spent organizing for participating dogs and owners, researchers etc, has been the foundation for all this - there would not have been any thesis without having you provide so much of the material. Thank you also for always making sure we had a bit of “the good things in life” making it worth while to spend week-ends and evenings doing research.

Professor Peter Lord, my co-supervisor and former head of Department. Having one of the real experts in thoracic radiology in the team has been invaluable. I have been spoiled having you just a few meters down the hall always allowing me to interrupt you with all sorts of questions. Thanks for guiding me through this last process of putting it all together, its been worth a million!

Associate professor Johan Carlsten, my initial supervisor. You finally managed to convince me to “give it a go” – thanks a lot. I hope you are still as enthusiastic as ever.

Henrik Duelund Pedersen, Olli Vuolteenaho, Kerstin Olsson and Anders Eriksson, co-authors and co-workers for sharing your expertise in your respective areas of research. Henrik and Anders for sharing the work with organizing hundreds of cavalier radiographs. Anders, for inventing the Eriksson index, sorry that it didn´t work out, but Br-Sp was great to have.

All the observers that so enthusiastically took part in my studies. No one named and no one forgotten. You gave me your time and a unique material. I am forever grateful to all of you. Thank you so much!!

The dogs and their owners, my gratitude is yours.

Chris Lombard, visiting professor during the 80´s, for introducing me to the mysteries of echocardiography.

Patrik Öhagen for excellent statistical consultation.

All of you in the “SVEP project”. It was great working together, I miss our yearly boat trips.

Per (XL), Maggi and Estelle and your respective families, friends and colleagues. You have all been around during this long journey, always having a minute to share, supporting me and taking over many of my responsibilities in clinics as well as in the rest of the work that needs to done. It is not everyone’s luck to work together with some of your best friends. Calle, for being the best godson a Madder can have.

Lotte, friend, travel guide, computer wizard – though sometimes things went wrong. Having you enthusiastically preceding me on this bumpy ride has been “supert” and encouraging. I still keep your supporting mail in my computer.

Henrik, Mats, Nalle and Suchi, former and present inmates in the room next door. Having you all as PhD friends and colleagues has been a great source of inspiration.

Joe, Barbara, Chris, John G, John F, Tobias, Sigridur, Mairi, Wil, Susi, Fintan, Marjatta, Hege, Giacomo, Federica – my international colleagues - I´ve probably forgotten some of you. Thanks for great friendship and introducing “world around cooking”. It has been a privileged working with you all.

All present and former veterinarians and technical staff at the department, Anders, Anna, Anna, Bettan, Charles, Emelie, Ewa, Ina, Jenni, Karina, Lillan, Madde, Marita, Mieth, Peter and Vivan. Thank you all for being such a nice bunch of people, for encouragement and support and for taking over some of my duties. I have a lot of lost rounds, journal clubs etc. to make up for. Thanks for providing the best of working atmospheres.

Ewa, many thanks for always taking your time with everything I needed and of course – a few giggles from Cyber space.

Mieth, friend and travel mate - there are still discoveries to make in the worlds of barley and grapes. Thanks for being there when “dynamics of life” hit me – we have seen a few sunrises together.

Anna-Karin, Anette, Karin, Kristina, Margareta and Marianne, for genuine friendship that lasted long beyond vet school. We need to make more time for the good things in life.

Karin S., for being the best of friends. You are my hero.

Sara, for being a great friend since 44 years in all kind of adventures, some still unknown to our parents. Your entire family has been a great support during the last years. Thank you all.

Britta, Gunnel and families Isberg, Lang and Geiger for continuing our family traditions. Nothing and everything has changed. I'm proud and grateful for having you as my sisters.

Kjell-Arne, for standing beside me in times of trouble, supporting, loving and always making me laugh. For letting me share your pearl - "the house by the waterfall to which no cell-phones reach"

Mum and dad – I wish you were here – in some way I'm sure you are

References

Barthez, P.Y., Hornof, W.J., Théon, A.P., Craychee, T.J. & Morgan, J.P. 1994. Receiver operating characteristic curve analysis of the performance of various radiographic protocols when screening dogs for pulmonary metastases. *Journal of the American Veterinary Medical Association* 204, 237-240.

Beardow, A.W. & Buchanan, J.W. 1993. Chronic mitral valve disease in cavalier King Charles spaniels: 95 cases (1987-1991). *Journal of the American Veterinary Medical Association* 203, 1023-1029.

Birkelo, C.C., Chamberlain, W.E. & Phelps, P.S. 1947. Tuberculosis case finding. A comparison of the effectiveness of various roentgenographic and photofluorographic methods. *Journal of American Medical Association* 133, 359-366.

Boon, J., Wingfield, W.E. & Miller, C.W. 1983. Echocardiographic indices in the normal dog. *Veterinary Radiology* 24, 214-221.

Bonagura, J.D., O´Grady, M.R. & Herring, D.S. 1985. Echocardiography: Principles of interpretation. *Veterinary Clinics of North America: Small Animal Practice* 15, 1177-1195.

Brown, O.R., Harrison, D.C. & Popp, R.L. 1974. An improved method for echocardiographic detection of left atrial enlargement. *Circulation* 50, 58-64.

Buchanan, J.W. 1968. Radiology of the heart. *Proceedings 35th Annual Meeting of American Animal Hospital Association.* 35-36.

Buchanan, J.W, & Bücheler, J. 1995. Vertebral scale system to measure canine heart size in radiographs. *Journal of the American Veterinary Medical Association* 206, 194–199.

Buchanan, J.W. 2000. Vertebral scale system to measure heart size in radiographs. *Veterinary Clinics of North America: Small Animal Practice* 30, 379–393.

Burnett, J.C., Kao, P.C., Hu, D.C., Heser, D.W., Heublein, D., Granger, J.P., Opgenorth, T.J. & Reeder, G.S. 1986. Atrial natriuretic peptide elevation in congestive heart failure in the human. *Science* 231, 1145-1147

Caylor, K.B., Zumpano, C.A., Evans, L.M. & Moore, R.W. 2001. Intra- and interobserver measurement variability of tibial plateau slope from lateral radiographs in dogs. *Journal of the American Hospital Association* 37, 263-268.

Centor, R.M. 1991. Signal detectability: the use of ROC curves and their analyses. *Medical Decision Making* 11, 102-106.

Christensen, E.E., Murry, R.C., Holland, K., Reynolds, J., Landay, M.J. & Moore, J.G. 1981. The effect of search time on perception. *Radiology* 138, 361-365.

Curry, T.S., Dowdey, J.E. & Murry, R.C. 1990. *Christensen´s Physics of Diagnostic Radiology.* Lippincott Williams & Wilkins. Philadelphia, USA, 323-371.
Darke, P.G.G. 1987. Valvular incompetence in cavalier King Charles spaniels. *The Veterinary Record* 120, 365-366.
Darke, P.G.G. 1995. Mitral valve disease in cavalier King Charles spaniels. In: Bonagura (Editor) *Kirk´s Current Veterinary Therapy, XII, Small Animal Practice*. W.B. Saunders, Philadelphia, USA, 837-841.
Das, K.M & Tashjian, R.J. 1965. Chronic mitral valve disease in the dog. *Veterinary Medicine/Small Animal Clinician* 60, 1209-1216.
Detweiler, D.K. & Patterson, D.F. 1965. The prevalence and types of cardiovascular disease in dogs. *Annals of the New York Academy of Science* 127, 481-516.
Detweiler, D.K., Luginbühl, H., Buchanan, J.W. & Patterson, D.F. 1968. The natural history of acquired cardiac disability of the dog. *Annals of the New York Academy of Science* 147, 318-329.
Ettinger, S.J. & Suter, P.F. 1970. *Canine cardiology.* W.B. Saunders. Philadelphia, USA, 321-382.
Fettig, A.A., Rand, W.M., Sato, A.F., Solano, M., McCarthy, R.J. & Boudrieau, R.J. 2003. Observer variability of tibial plateau slope measurement in 40 dogs with cranial cruciate ligament-deficient stifle joints. *Veterinary Surgery* 32, 471-478.
Fox, P.R., Sisson, D. & Moise, N.S. (Editors). 1999. Appendix A.: Recommendations for diagnosis of heart disease and treatment of heart failure in small animals. *Textbook of canine and feline cardiology*. 2nd edition., W.B. Saunders, Philadelphia, USA.
Grossmann, G., Giesler, M., Schmidt, A., Kochs, M., Wieshammer, S., Felder, C., Hoher, M. & Hombach, V. 1995. Assessment of severity of mitral insufficiency – Value of various color Doppler echocardiographic methods. *Z Kardiologie* 84, 190-197.
Hamlin, R.L. 1968. Analysis of the cardiac silhouette in dorsoventral radiographs from dogs with heart disease*. Journal of the American Veterinary Medical Association* 153, 1444-1460.
Hanley, J.A. 1989. Receiver operating characteristic (ROC) methodology: The state of the art. *Critical Reviews in Diagnostic Imaging* 29, 307-335.
Hansson, K., Häggström, J., Kvart, C. & Lord, P.F. 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. *Veterinary Radiology & Ultrasound* 43, 568-575.
Herman, P.G. & Hessel, S.J. 1975. Accuracy and its relationship to experience in the interpretation of chest radiographs. *Investigative Radiology* 10, 62-67.
Hessel, S.J., Hermann, P.G. & Swensson, R.G. 1978. Improving performance by multiple interpretations of chest radiographs: effectiveness and cost. *Radiology* 127, 589-594.
Holmer, Riegger & Notheis, W.F, Kromer, E.P. & Kochsiek, K. 1987. Hemodynamic changes and renal plasma flow in early heart failure:implications for renin, aldosterone, norepinephrine, atrial natriuretic peptide and prostacyclin. *Basic Research in Cardiology* 82, 101-108.
Häggström, J., Hansson, K., Kvart, C. & Swenson, L. 1992. Chronic valvular disease in the cavalier King Charles spaniel in Sweden. *The Veterinary Record* 131, 549-553.
Häggström, J., Hansson, K., Karlberg, B.E., Kvart, C. & Olsson, K. 1994. Plasma concentration of atrial natriuretic peptide in relation to severity of mitral regurgitation in Cavalier King Charles Spaniels. *American Journal of Veterinary Research* 55, 698-703.
Häggström, J., Hansson, K., Karlberg, B.E., Kvart, C., Madej, A. & Olsson, K. 1996. Effects of long-term treatment with enalapril or hydralazine on the renin-angiotensin-aldosterone system and fluid balance in dogs with naturally acquired mitral valve regurgitation. *American Journal of Veterinary Research* 57, 1645-1652.
Häggström, J., Hansson, K., Kvart, C., Karlberg, B.E., Vuolteenaho, O. & Olsson, K. 1997. Effects of naturally acquired decompensated mitral valve regurgitation on renin-angiotensin-aldosterone system and natriuretic peptide concentration in dogs. *American Journal of Veterinary Research* 58, 77-82.
Häggström, J., Hansson, K., Kvart, C., Vuolteenaho, O. & Olsson, K. 2000. Relationship between different natriuretic peptides and severity of naturally acquired mitral

regurgitation in dogs with myxomatous valve disease. *Journal of Veterinary Cardiology* 1, 7-16.

Kealy, J.K. 1979. *Diagnostic radiology of the dog and cat.* W.B. Saunders, Philadelphia, 215-216.

Kienle, R.D. & Thomas, W.P. 2002. Echocardiography. In: Nyland, T.G. & Mattoon, J.S (Editors). *Small Animal Diagnostic Ultrasound*. 2nd edition. W.B. Saunders Company, Philadelphia, USA, 354-423.

Kittleson, M.D. & Brown, W.A. 2003. Regurgitant fraction measured by using the proximal isovelocity surface area method in dogs with chronic myxomatous mitral valve disease. *Journal of Veterinary Internal Medicine* 17, 84-88.

Kvart, C., Häggström, J., Pedersen, H.D., Hansson, K., Eriksson, A., Järvinen, A-K., Tidholm, A., Bsenko, K., Ahlgren, E., Ilves, M., Åblad, B., Falk, T., Bjerkås, E., Gundler, S., Lord, P., Wegeland, G., Adolfsson, E. & Corfitzen, J. 2002. Efficacy of enalapril for prevention of congestive heart failure in dogs with myxomatous valve disease and asymptomatic mitral regurgitation*. Journal of Veterinary Internal Medicine* 16, 80-88.

Lamb, C.R., Tyler, M., Boswood, A., Skelly, B.J. & Cain, M. 2000. Assessment of the value of the vertebral heart scale in the radiographic diagnosis of cardiac disease in dogs. *The Veterinary Record* 146, 687-690.

Lamb, C.R., Wikeley, H., Boswood, A. & Pfeiffer, D.U. 2001. Use of breed-specific ranges for the vertebral heart scale as an aid to the radiographic diagnosis of cardiac disease in dogs. *The Veterinary Record* 148, 707–711.

Lehmkuhl, L.B., Bonagura, J.D., Biller, D.S. & Hartman, W.M. 1997. Radiographic evaluation of caudal vena cava size in dogs. *Veterinary Radiology & Ultrasound* 38, 94-100.

Litster, A.L. & Buchanan, J.W. 2000a. Vertebral scale system to measure heart size in radiographs of cats. *Journal of the American Veterinary Medical Association* 216, 210–214.

Litster, A.L. & Buchanan, J.W. 2000b. Radiographic and echocardiographic measurement of the heart in obese cats. *Veterinary Radiology & Ultrasound* 41, 320-325.

Lombard, C. 1984. Normal values of the canine M-mode echocardiogram. *American Journal of Veterinary Research* 45, 2015-2018.

Lombard, C.W. & Spencer, C.P. 1985. Correlation of radiographic, echocardiographic, and electrocardiographic signs of left heart enlargement in dogs with mitral regurgitation. *Veterinary Radiology* 26, 89-97.

Lord, P.F. 1975. Cardiac mensuration. In: Kirk, R.W (Editor) *Current Veterinary Theraphy V: Small Animal Practice.* W.B. Saunders, Philadelphia, USA, 339-340.

Lusted, L.B. 1960. Logical analysis in roentgen diagnosis. *Radiology* 74, 178-193.

Melián, C., Stefanacci, J., Peterson, M.E. & Kintzer, P.P. 1999. Radiogrphic findings in dogs with naturally-occuring primary hypoadrenocorticism. *Journal of the American Animal Hospital Association* 35, 208-212.

Metz, C.E. 1978. Basic principles of ROC analysis. *Seminars in Nuclear Medicine* 8, 283-298.

Morrison, S.A., Moise, N.S., Scarlett, J., Mohammed, H. & Yeager, A. 1992. Effects of breed and body weight on echocardiographic values in four breeds of dogs of differing somatotype. *Journal of Veterinary Internal Medicine 6, 220-224.*

Nakayama, H., Nakayama, T. & Hamlin, R.L. 2001. Correlation of cardiac enlargement as assessed by vertebral heart size and echocardiographic and electrocardiographic findings in dogs with evolving cardiomegaly due to rapid ventricular pacing. *Journal of Veterinary Internal Medicine* 15, 217–221.

Nørgaard, H., Gjørup, T., Brems-Dalgaard, E., Hartelius, H. & Brun, B. 1990. Interobserver variation in the detection of pulmonary venous hypertension in chest radiographs. *European Journal of Radiology* 11, 203-206.

Pedersen, H.D., Koch, J., Poulsen, K., Jensen, A.L. & Flagstad, A. 1995. Activation of the renin-angiotensin system in dogs with asymptomatic and mildly symptomatic mitral valvular insufficiency. *Journal of Veterinary Internal Medicine* 9, 328-331.

Pedersen, H.D., Lorentzen, K.A. & Kristensen, B.Ø. 1996. Observer variation in the two-dimensional echocardiographic evaluation of mitral valve prolapse in dogs. *Veterinary Radiology & Ultrasound* 37, 367-372.
Pedersen, H.D., Lorentzen, K.A. & Kristensen, B.Ø. 1999. Echocardiographic mitral valve prolapse in cavalier King Charles spaniels: epidemiology and prognostic significance for regurgitation. *The Veterinary Record* 144, 315-320.
Perkins, R.L. 1979. Effect of radiographic positioning on the heart shadow in dogs. *Modern Veterinary Practice* 60, 801-805.
Pistolesi, M., Miniati, M., Milne, E.N.C. & Giuntini, C. 1985. The chest roentgenogram in pulmonary edema. *Clinics in Chest Medicine* 6, 315-344.
Potchen, J.E., Cooper, T.G., Sierra, A.E., Aben, G.R., Potchen, M.J., Potter, M.G. & Siebert, J.E. 2000. Measuring performance in chest radiography. *Radiology* 217, 456-459.
Quekel, L.G., Kessels, A.G., Goei, R. & van Engelshoven, J.M. 2001. Detection of lung cancer on chest radiography: a study on observer performance. *European Journal of Radiology* 39, 111-116.
Rackow, P.L., Spitzer, V.M. & Hendee, W.R. 1987. Detection of low-contrast signals. A comparison of observers with and without radiology training. *Investigative Radiology* 22, 311-314.
Riegger, Liebau & Holzschuh, M., Witkowski, D., Steilner, H. & Kochsiek, K. 1984. Role of the renin-angiotensin system in the development of congestive heart failure in the dog as assessed by chronic converting enzyme blockade. *American Journal of Cardiology* 53, 614-618.
Riegger, A.J. 1990. Interaction between atrial natriuretic peptide, renin system and vasopressin in heart failure. *European Heart Journal* 11, 79-83.
Rishniw, M. & Erb, H.N. 2000. Evaluation of four 2-dimensional echocardiographic methods of assessing left atrial size in dogs. *Journal of Veterinary Internal Medicine* 14, 429-435.
Robinson, P.J.A., Wilson, D., Coral, A., Murphy, A. & Verow, P. 1999. Variation between experienced observers in the interpretation of accident and emergency radiographs. *The British Journal of Radiology* 72, 323-330.
Root, C.R. & Bahr, R.J. 2002. The heart and great vessels. In: Thrall D.E. (Editor). *Textbook of Veterinary Diagnostic Radiology*. 4th edition. W.B. Saunders Company, Philadelphia, USA, 402-419.
Ruehl, W.W. & Thrall, D.E. 1981. The effect of dorsal versus ventral recumbency on the radiographic appearance of the canine thorax. *Veterinary Radiology* 22, 10-16.
Schelling, C.G. 1995. Radiology of the heart. In: Miller, M.S. & Tilley, L.P. (Editors) *Manual of Canine and Feline Cardiology*. 2nd edition. W.B. Saunders, Philadelphia, USA, 17-45.
Schulze, W. & Nöldner, H. 1957. Röntgenologische fernaufnahmen des hundeherzens und versuch ihrer deutung mit hilfe einer linearen mezmethode. *Aus der Klinik und Poliklinik für kleine haustiere der Karl-Marx-Universität,* Leipzig, 442-458.
Silverman, S. & Suter, P.F. 1975 Influence of inspiration and expiration on canine thoracic radiographs. *Journal of the American Veterinary Medical Association* 166, 502–510.
Sleeper, M.M. & Buchanan, J.W. 2001. Vertebral scale system to measure heart size in growing puppies. *Journal of the American Veterinary Medical Association* 219, 57-59.
Snashall, P.D., Keyes, S.J., Morgan, B.M., McAnulty, R.J., Mitchell-Heggs, P.F., McIvor, J.M. & Howlett, K.A. 1981. The radiographic detection of acute pulmonary oedema. A comparison of radiographic appearances, densitometry and lung water in dogs. *British Journal of Radiology* 54, 277-288.
Staub, N.C. 1974. Pulmonary edema. *Physiological Reviews* 54, 670-811.
Suter, P.F. & Lord, P.F. 1984. *Thoracic radiography: A text atlas of thoracic diseases of the dog and cat*. Selbstverlag PF Suter, Wettswil, Switzerland.
Tan, L., Jalil, J., Pick, R., Janicki, J.S. & Weber, K.T. 1991. Cardiac myocyte necrosis induced by angiotensin II. *Circulatory Research* 69, 1185-1191.
Teele, R.L. & Griscom, N.T. 1998. Aunt Minnie (letter). *Radiology* 208, 829-830.
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 the cat. *Journal of Veterinary Internal Medicine* 7, 247-252.

Toombs, J.P. & Ogburn, P.N. 1985. Evaluating canine cardiovascular silhouettes: radiographic methods and normal radiographic anatomy. *The Compendium on Continuing Education for the Practicing Veterinarian* 7, 579-587.

Uhlig, K. & Werner, J. 1969. Eine roentgenographische methode zur messung der herzvergroesserung beim hund. *Berliner und Münchener Tierärztliche Wochenschrift* 6, 110-112.

Utsunomiya, T. Ogawa, T., Tang, H.A., Doshi, R., Patel, D., Quan, M., Henry, W.L. & Gardin, J.M. 1991. Doppler color flow mapping of the proximal isovelocity surface area: a new method for measuring volume flow rate across a narrow orifice. *Journal of American Society of Echocardiography* 4, 338-348.

van Der Heijde, D., Boonen, A., Boers M., Kostense, P. & van Der Linden, S. 1999. Reading radiographs in chronological order, in pairs or as single films has important clinical implications for the discriminative power of rheumatoid arthritis clinical trials. *Rheumatology* 38, 1213-1220.

Vining, D.J. & Gladish, G.W. 1992. Receiver operating characteristic curves: A basic understanding. *RadioGraphics* 12, 1147-1154.

Vuolteenaho, O., Arjamaa, O. & Ling, N. 1985. Atrial natriuretic polypeptides (ANP): rat atria store high molecular weight precursor but secrete processed peptides of 25-35 amino acids. *Biochemical and Biophysical Research Communications* 129, 82-88.

Wei, C.M., Heublein, D.M., Perrella, M.A., Lerman, A., Rodeheffer, R.J., McGregor, C.G., Edwards, W.D., Schaff, H.V. & Burnett, J.C. 1993. Natriuretic peptide system in human heart failure. *Circulation* 88, 1004-1009.

Zontine, W.J. 1975. Bronchography in the dog. In: Ticer, J.W. (Editor). *Radiographic technique in small animal practice.* W.B. Saunders Company, Philadelphia, USA, 315-323.