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Canine Idiopathic Dilated Cardiomyopathy

Epidemiology, histopathology and pathophysiology

Anna Tidholm

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

Dilated cardiomyopathy (DCM), one of the most common heart diseases in dogs, is a disease of unknown etiology characterized by chamber dilatation and myocardial systolic and diastolic dysfunction, ultimately leading to congestive heart failure (CHF). Since the advent of echocardiography, myocardial hypokinesis and severe left atrial and ventricular dilatation without other detectable cardiac abnormalities have been regarded as diagnostic criteria for DCM. Canine DCM is often referred to as being breed specific. Reports on histologic characterization of DCM are scant in breeds other than the Doberman pinscher.

The aims of the studies presented here were (1) to characterize the clinical, electrocardiographic, radiographic, echocardiographic, and pathologic findings in a homogeneous (Newfoundlands) population and in a large, and with respect to breed heterogeneous, (comprising 38 breeds) population of dogs with DCM, (2) to estimate survival times and identify prognostic indicators for dogs presenting with CHF caused by DCM, (3) to determine the prevalence of attenuated wavy fibers in the myocardium of dogs and the sensitivity and specificity of this finding for DCM, (4) to determine the sensitivity of standard clinical-echocardiographic criteria for DCM, using post mortem findings for final diagnosis, (5) to determine the sequence of events regarding the development of attenuated wavy fibers in relation to echocardiographic evidence of chamber dilatation and myocardial hypokinesis in DCM, (6) to investigate the activity of the renin-angiotensin-aldosterone system (RAAS) and atrial natriuretic peptide (ANP) in dogs with asymptomatic and symptomatic DCM, and (7) to determine concentrations of circulating thyroid hormones in DCM, and whether the expression of mRNA coding for thyroid hormone receptors was altered in dogs with CHF due to DCM in comparison to dogs in CHF due to chronic valvular disease (CVD).

There were no major differences concerning clinical, electrocardiographic, radiographic, echocardiographic and histopathologic characteristics between the homogeneous group, i.e. Newfoundlands, and the heterogeneous group of dogs. DCM carries a poor prognosis in dogs, and only age at time of diagnosis and the presence of dyspnea or ascites can be used as prognostic markers. Young age at onset of clinical signs was the most significant risk factor identified.

The histologic finding of attenuated wavy fibers has a very high sensitivity (99%) and specificity (100%) for canine idiopathic DCM. Attenuated wavy fibers may develop before any clinical or echocardiographic signs of heart disease are evident, thus indicating an early stage of DCM, which may be denoted "occult DCM". Sensitivity of standard clinical and echocardiographic criteria for the diagnosis of DCM is 93%, when the final diagnosis is based on post mortem findings.

The RAAS and ANP concentrations were significantly increased in dogs with clinical signs of DCM, but not in dogs with subclinical DCM. Total thyroxine and triiodothyronine concentrations were not decreased in a majority of dogs with CHF caused by DCM. However, free thyroxine concentrations were significantly decreased in dogs with symptomatic DCM, compared to dogs with asymptomatic DCM and to normal control dogs. Messenger RNA for thyroid hormone receptor subtypes ß1 and ß2 was upregulated in dogs with CHF attributable to DCM or CVD.

Key words: dog, heart disease, dilated cardiomyopathy, asymptomatic dilated cardiomyopathy, epidemiology, histopathology, attenuated wavy fibers, thyroid hormone receptor, renin, angiotensin II, aldosterone. atrial natriuretic peptide.

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"Above stands the marble smile of the implacable Nature which has endowed us more with longing than with intellectual capacity"

Albert Einstein 1923

To Gunnar, Nisse and Erika

ABSTRACT

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HJÄRTMUSKELSJUKDOM HOS HUND

Sammanfattning

Hjärtmuskelsjukdomar kan indelas i primära och sekundära hjärtmuskelsjukdomar, där de förra omfattar sjukdomar som uppstår primärt i hjärtat, ofta utan känd orsak, medan de senare orsakas av t.ex. inflammationer, toxiner, ämnesomsättningsrubbningar eller förändringar i blodcirkulationen. Primära hjärtsjukdomar kan i sin tur indelas i dilaterad cardiomyopati (DCM, hjärtmuskelsvaghet), där hjärtmuskelns sammandragningsförmåga är starkt nedsatt, och hypertrofisk eller restriktiv cardiomyopati (hjärtmuskelförtjockning resp. hjärtmuskelstelhet), där hjärtats förmåga att fyllas mellan hjärtslagen är nedsatt. Dilaterad cardiomyopati är den vanligast förekommande formen av hjärtmuskelsjukdom hos hundar, och många olika medelstora och stora hundraser drabbas. Symptom på sjukdomen omfattar andningssvårigheter, trötthet, försämrad aptit och hosta, och orsakas av hjärtsvikt, d.v.s. vätskeutträde i lungorna (lungödem), lungsäcken, hjärtsäcken eller bukhålan. Medelålder för symptomdebuten är ca. 5 år, men även valpar och unghundar kan insjukna. För att fastställa diagnosen krävs ultraljudsundersökning av hjärtat, s.k. ekokardiografi, där den nedsatta pumpförmågan och förstoring av hjärtats hålrum kan ses.

Då andra faktorer utöver DCM kan orsaka hjärtmuskelsvaghet och hjärtförstoring, visar studierna i denna avhandling att obduktion med mikroskopisk bedömning krävs för slutgiltig diagnos. Resultaten visar också att de mikroskopiska förändringarna i hjärtmuskeln vid DCM är mycket typiska för, och uteslutande ses vid, DCM, samt att dessa förändringar uppträder innan sjukdomen kan upptäckas kliniskt eller med ekokardiografi.

Prognosen vid DCM är mycket varierande, och svår att förutsäga i det enskilda fallet. En del hundar avlivas omgående av djurskyddsskäl pga svår hjärtsvikt, medan andra blir i stort sett symptomfria med medicinsk behandling, och kan leva så i flera år. Dödligheten under första året, efter att diagnosen fastställts med ekokardiografi, är dock generellt sett hög (82.5 %). Att kunna förutsäga prognosen i det enskilda fallet vore av stort värde, men visar sig vara svårt. Förutom sjukdomens svårighetsgrad, visade sig endast låg ålder vid insjuknandet vara av negativ prognostisk betydelse.

Kroppens olika hormonsystem, ämnade att bevara hjärtfunktionen vid t.ex. blodförlust och cirkulatorisk kollaps, aktiveras vid manifest hjärtsvikt. Dessa försvarssystem ökar dock ytterligare belastningen på hjärtmuskeln genom att öka blodvolymen och blodtrycket, och påskyndar och förvärrar därmed hjärtsvikten. Medicinsk behandling av hjärtsvikt syftar till att hämma kroppens aktivering av nämnda försvarssystem. Resultaten i föreliggande avhandling visar att hormonsystemen är aktiverade hos hundar med symptom på hjärtsvikt, men ej hos hundar med enbart ekokardiografiska tecken på DCM. Då vi endast kan mäta blodnivåer av hormoner, och det även finns lokalt i hjärtmuskeln producerade hormoner, kan vi sannolikt endast genom kliniska behandlingsstudier fastställa i vilket stadium av hjärtsjukdom medicinsk intervention är indicerad.

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APPENDIX

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

I. Tidholm A, Jönsson L. Dilated cardiomyopathy in the Newfoundland: A study of 37 cases (1983-1994). J Am Anim Hosp Assoc 1996;32:465-470

II. Tidholm A, Jönsson L. A retrospective study of canine dilated cardiomyopathy (189 cases). J Am Anim Hosp Assoc 1997;33:544-550

III. Tidholm A, Svensson H, Sylvén C. Survival and prognostic factors in 189 dogs with dilated cardiomyopathy. J Am Anim Hosp Assoc 1997;33:364-368

IV. Tidholm A, Häggström J, Jönsson L. Prevalence of attenuated wavy fibers in myocardium of dogs with dilated cardiomyopathy. J Am Vet Med Assoc 1998;202:1732-1734

V. Tidholm A, Häggström J, Jönsson L. Detection of attenuated wavy fibers in the myocardium of Newfoundlands without clinical or echocardiographic evidence of heart disease. Am J Vet Res 2000;61:238-241

VI. Tidholm A, Häggström J, Hansson K. Effects of naturally occurring symptomatic and asymptomatic dilated cardiomyopathy on the renin-angiotensin-aldosterone system, atrial natriuretic peptide and thyroid hormone concentrations in dogs. Am J Vet Res., accepted for publication

VII. Shahrara S, Tidholm A, Drvota V, Häggström J, Sylvén C. Upregulation of thyroid hormone receptor ß1 and ß2 messenger RNA in the myocardium of dogs with dilated cardiomyopathy or chronic valvular disease. Am J Vet Res 1999;60:848-852

ABBREVIATIONS

The following abbreviations are used in the text:

2D	two dimensional echocardiography
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
AII	angiotensin II
ANP	atrial natriuretic peptide
Ao	aorta
ATP	adenosine triphosphate
ATP	aseadenosine triphosphatase
BNP	brain natriuretic peptide
C-terminal	carboxy terminal
cAMP	cyclic adenosine monophophate
CHF	congestive heart failure
CVD	chronic valvular disease
DCM	dilated cardiomyopathy
DNA	deoxyribonucleic acid
ECG	electrocardiography
ELISA	enzyme-linked immunosorbent assay
EPSS	E-point septal separation
FS	fractional shortening
FT4	free thyroxine
H&E	hematoxylin eosin
HC	hypertrophic cardiomyopathy
HL	human leukocyte antigen
ISACH	The International Small Animal Cardiac Health Council
LA	left atrium
LVEDD	left ventricular end diastolic diameter
LWmean	left ventricular wall mean thickness
M-mode	motion mode
MHC	myosin heavy chain
MR	mitral regurgitation
mRNA	messenger ribonucleic acid
NADH	nicotinamide adenine dinucleotide (reduced form)
NT-proANP	N-terminal pro-atrial natriuretic peptide
NYHA	New York Heart Association
PAC	plasma aldosterone concentration
pap.m.	papillary muscle
PRA	plasma renin activity
RAAS	renin angiotensin aldosterone system
RNA	ribonucleic acid
S3	third heart sound
T3	3,5,3′-triiodothyronine
TR	thyroid hormone receptor
TSH	thyroid stimulating hormone

TT4	total thyroxine
tRNA	transfer RNA
VPD	ventricular premature depolarizations

INTRODUCTION

1. Definitions and clinical characteristics

The term cardiomyopathy was coined by Bridgen in 1957 to describe myocardial disease, not attributable to coronary-artery disease, rheumatic or infectious disease, hypertension, or valvular disease.[1] The subsequent division of cardiomyopathies in three major categories has been described by several authors.[2-5] These are (1) cardiac dilatation with atrio-ventricular incompetence and congestive heart failure, i.e. congestive, or dilated, cardiomyopathy (DCM), (2) cardiac hypertrophy with or without obstruction, i.e. hypertrophic cardiomyopathy, and (3) signs of cardiac constriction, i.e. restrictive cardiomyopathy. This classification of cardiomyopathies was promulgated in 1980 by the World Health Organization (WHO)[6,7] and is now widely recognized. Dilated idiopathic cardiomyopathy, which is the most prevalent form of cardiomyopathy in dogs,[8-10] was first reported in this species in 1970, as congestive heart failure in conjunction with dilatation of the cardiac chambers and absence of other clinically important cardiovascular disease by Ettinger, Bolton and Lord,[11] and by others.[11] Since the advent of echocardiograhy, myocardial hypokinesis measured as low FS and severe left atrial and ventricular dilatation without other detectable cardiac abnormalities have been regarded as diagnostic criteria for DCM.[8,12,13]

Primary, or idiopathic, DCM is by definition of unknown or uncertain etiology and not the result of an identifiable systemic disorder or cardiovascular disease.[14] Secondary cardiomyopathies are classified according to the etiology as drug- or toxin-induced, genetic, infiltrative, ischemic, metabolic, nutritional, or inflammatory myocardial diseases.[15] Subclinical, or asymptomatic, cardiomyopathy is commonly defined as the condition where there is echocardiographic evidence of left ventricular dilatation and hypokinesis in the absence of clinical and radiographic evidence of congestive heart failure.[16-20] Canine dilated cardiomyopathy is often referred to as being breed-specific for Doberman pinschers, boxers, English cocker spaniels, and lately also for Portuguese water dogs.[15,21] Harpster has described distinct histologic characteristics in a group of boxers, i.e. boxer cardiomyopathy.[22]

Clinical presentation of DCM includes signs of left-sided or biventricular congestive heart failure, i.e. pulmonary edema, pleural effusion, or ascites. A soft, regurgitant, systolic murmur is sometimes audible over the mitral valve region, along with a low-pitched protodiastolic (S3) gallop sound, as evidence of severe ventricular impairment.[15,23,24] Atrial fibrillation is the most commonly diagnosed electrocardiographic abnormality in DCM,[10,25,26] although ventricular premature depolarizations and ventricular tachycardia are reported in a majority of Doberman pinschers.[27,28] Harpster reported on boxer cardiomyopathy with three different clinical categories, ranging from asymptomatic arrhythmias to congestive heart failure.[22] Echocardiographic

evaluation of left ventricular systolic performance reveals increased end-systolic and end-diastolic dimensions and decreased FS, as well as changes in systolic time intervals.[29-32] Diastolic dysfunction, as evidenced by Doppler examination derived prolonged relaxation time and left ventricular inflow pattern of increased early diastolic to atrial wave ratio (E/A), has been correlated to clinical deterioration in human patients with DCM.[33,34] Left ventricular diastolic stiffness, estimated by cineangiographic derived pressure-volume loops, was reported in dogs with DCM in 1976 by Lord.[35] An index of combined systolic and diastolic myocardial performance has been advocated in human patients as a measure of cardiac function in DCM.[36]

2. Etiology and pathogenesis

The etiology of idiopathic dilated cardiomyopathy is by definition uncertain or not known. There are, however, a diverse spectrum of suspected and known causes of myocardial hypokinesis which will be discussed in this context. Proposed causes of DCM can be included in seven major categories: familial and genetic factors, nutritional deficiencies, metabolic disorders, immunologic abnormalities, infectious diseases, drug-and toxin-induced, and tachycardia-induced cardiomyopathies. In a study of 673 human patients clinically diagnosed with DCM, 47% were classified as idiopathic, 12% caused by myocarditis, 11% caused by coronary artery disease, and 31% of other identifiable causes, based on histologic examination of endomyocardial biopsies of the right ventricle.[37]

Dilated cardiomyopathy is considered to be hereditary in approximately 20 to 35% of human patients.[38,39] Maternally inherited myopathy and cardiomyopathy was associated with mutations in mitochondrial DNA coding for tRNA leucin in one study.[40] The role of cytoskeletal proteins has been investigated in the development of cardiomyopathies, and the dystrophin gene has been identified as being responsible for X-linked dilated cardiomyopathy.[41,42] Canine X-linked muscular dystrophy may cause severe cardiac involvement,[43] and deletion of the entire dystrophin gene has been demonstrated in German short-haired pointers with skeletal myopathy and dilated cardiomyopathy.[44] Mutations in other cytoskeletal proteins, such as metavinculin, α-dystroglycan, α- and γ-sarcoglycan, and muscle LIM protein (a zinc finger structure, critical in maintaining the structural integrity of the contractile apparatus) may also result in DCM.[45] It has recently been shown that a heritable form of DCM in humans is caused by mutations of the actin gene.[46] Both dystrophin and actin are involved in force transmission by linking the cytoskeleton to the extracellular matrix, rather than force generation. In dogs, the fact that DCM is more prevalent in certain breeds or families of dogs suggests a genetic basis, although evidence is lacking. Molecular analysis of the cardiac actin gene in 16 Doberman pinschers with DCM did not reveal any abnormalities.[47]

Nutritional abnormalities causing myocardial hypokinesis, i.e. carnitine and/or taurine deficiencies, have been described in humans,[48] dogs,[49-53] and cats.[54] Long-chain fatty acids, which are quantitatively the most important energy-producing substrate of the myocardium, must be esterified to L-carnitine to be able to pass the mitochondrial membrane for subsequent ß-oxidation. Keene *et al.* have demonstrated low myocardial carnitine concentrations in some breeds with DCM, notably boxers, Doberman pinschers, and American cocker spaniels.[50,51,53] As myocardial carnitine levels are reduced in a majority of cardiac disorders in humans,[48] this finding may not be specific for DCM, but rather a consequence of congestive heart failure. Taurine is the most abundant free amino acid in the heart, and is known to regulate calcium influx across membranes in excitable tissues, such as nervous and muscle tissue.[55] A reversible dilated cardiomyopathy associated with low plasma taurine levels was reported in cats in 1987.[54] One study of DCM in cocker spaniels showed improvement of myocardial function following supplementation with taurine and L-carnitine.[53]

Metabolic disorders associated with DCM include diabetes mellitus, pheochromocytoma, and hypothyroidism.[14,15,56] The biologically active thyroid hormone T3 regulates genes coding for proteins essential for myocardial performance, i.e. Na,K-ATPase, Ca-ATPase, ß-adrenergic receptors and myosin heavy chains.[57-59] Release of ANP is inhibited,[60] and systolic time intervals may be reversibly changed in hypothyroidism.[61] The inotropic state of the left ventricle seems to vary directly with the thyroid state in experimental conditions in dogs,[62] and in thyroid hormone supplemented hypothyroid dogs,[63] whereas administration of thyroid hormones to euthyroid dogs did not influence echocardiographic measurements in other studies.[64,65] Serum concentrations of thyroid hormones or TSH do not seem to be changed in a majority of dogs with DCM,[27,66,67] and there appeared to be no relationship between cardiac status and hypothyroidism in a recent study of Doberman pinschers with DCM.[68]

Immunologic processes may be involved in the pathogenesis of DCM, as auto-antibodies have been detected against the cardiac ß-receptor,[69,70] the mitochondria,[71] the mitochondrial ADP/ATP translocator,[72,73] and α- and ß-myosin heavy chains,[74] either in humans or in experimental animals. Such antibodies are in many of these reports not restricted to DCM, but are also detected in myocarditis, hypertrophic cardiomyopathy and hypertensive heart disease.[75] Circulating heart-reactive antibodies were detected in humans with DCM or myocarditis, but not in normal individuals.[76,77] There were however no detectable differences between the amount of circulating antimyocardial antibodies in a study of normal dogs compared to dogs with DCM.[78] In yet another study, 30% of dogs with DCM had anti-mitochondrial antibodies.[79] HLA-antigens may be a genetic marker for susceptibility to DCM in humans.[80] An excessive immunologic reaction is also suggested in human DCM by increase of numbers of helper-T-cells,[81] and by increased T-cell activation.[82] Although these findings suggest that immunological processes

are involved in the pathogenesis of DCM as well as in other cardiac diseases, they do not elucidate the sequence of events concerning myocardial damage and immunologic activation.

It is well accepted that acute myocarditis can progress to a state of myocardial hypokinesis. Depending on report, DCM in human patients is thought to originate from myocarditis, as defined by the Dallas criteria, in 0 and 67%.[83-86] Inflammatory response to infectious agents, such as viruses,[87-90] bacteria,[91-93] and protozoa, e.g. Trypanosoma[94-96] has been proposed in the pathogenesis of myocarditis and DCM. Antibodies against α-helical structures of bacteria or viral antigens can lead to cytotoxic reactions which may explain the origin of some autoimmune cardiac diseases.[91] Antibodies against viral particles[89,90] and detection of virus RNA in myocardial biopsies from Coxsackie B virus,[87] as well as other enteroviruses,[88] have been documented in human patients with DCM or myocarditis. Parvovirus induced myocarditis in association with cardiomegaly and CHF has been reported in dogs,[97-99] and cardiac necrosis was induced in dogs experimentally infected with canine distemper virus.[100] It has been suggested that the evolution of myocardial hypokinesis following viral infection is predominantly autoimmune in nature, resulting from either shared antigens or molecular mimicry.[101]

Drug- and toxin-induced cardiomyopathies include cardiotoxicity caused by doxorubicin and other anti-neoplastic agents, ethanol, cobalt, lead, catecholamines, histamine, methylzantines, and vitamin D. Due to the broad spectrum of cardiotoxic agents, many different pathologic alterations of the myocardium are found.[102] For example, vacuolization of myocytes and necrosis is often detected in doxorubicin induced cardiomyopathy, and fatty infiltration is a major finding in ethanol induced cardiomyopathy.[103]

Left ventricular function is greatly influenced by heart rate. Several studies on experimentally induced tachycardia (>200 beats/min) report development of CHF and chamber dilatation, and reversible hypokinesis in dogs.[104-106] Ventricular tachycardia induces more profound changes in ejection fraction and left ventricular diastolic dimensions than does supraventricular tachycardia.[107] Non-induced tachycardia can also result in reversible myocardial dysfunction in both humans and dogs.[108,109] Pathophysiologic mechanisms involved in tachycardia-induced myocardial failure include increased oxygen demand, increased capillary-myocyte distance,[110] myocyte cell loss, elongation and hypertrophy of myocytes[111,112] decreased production of cAMP and abnormal calcium handling,[113] and alterations in left ventricular norepinephrine concentrations and sarcoplasmic reticular and myofibrillar CaATP-ase. These mechanisms are similar to those reported for cardiac volume and pressure overload.[104]

3. Pathophysiology and biochemical alterations

Contractility is the inherent property of muscle cells which determines the peak tension that can be developed starting from a specific resting fiber length.[114] The force of ventricular contraction depends on myocardial contractility, preload, i.e. end-diastolic volume, and the afterload, i.e. the interference to ejection of blood from the ventricle into the aorta.[115] Inability to generate a forceful contraction may be attributed to failure in any number of steps, from depolarization of the myocytes to contraction and relaxation of the myofibrils. Causal factors include electrolyte imbalance or changes in membrane permeability, reduction of calcium ion concentration outside the cell or a failure of normal opening of the calcium channels in the cell membrane, calcium ions too tightly bound to the sarcoplasmatic reticulum or to mitochondria, reduction of ATPase activity, and structural changes in contractile proteins. Myocardial relaxation is a highly energy-dependent process, and any of the factors involved in reduced contractility may also cause relaxation to falter.[116]

Several reports on the pathophysiology of DCM address the inadequate ATP-production within the mitochondria. Impaired oxidative production of ATP has been documented in Doberman pinschers with DCM associated with respiratory chain defects (60% reduction of NADH dehydrogenase and 50% reduction of ATP syntethase), and reduction of myoglobin concentrations.[117-119] Reduction of myoglobin was also detected in induced myocardial failure in various animal models.[118] Reduction of cytosolic calcium may be caused by abnormalities of cAMP production,[113] and abnormal calcium release channel activity, features that have been documented in humans with DCM,[120] and in dogs with spontaneous DCM or pacing induced hypocontractility.[121] Carnitine deficiency, and/or decreased amounts of acetyl coenzyme A from ß-oxidation of free fatty acids entering the mitochondria, will lead to decreased production of ATP. Increased concentration of the antioxidant glutathione peroxidase and a negative correlation between disease severity and vitamin E concentration in dogs with DCM may suggest involvement of the oxidant-antioxidant system.[122] Increased activity of the proteolytic enzymes promatrix metalloproteinase-9 and neutrophil elastase in Doberman pinschers in particular with, but also without, DCM may implicate an inflammatory response in the pathogenesis of DCM, as well as a breed specific predisposition for the disease.[123] It is not clear whether observed biochemical alterations are causing myocardial dysfunction or are adaptive changes to, or a consequence of, heart failure.

4. Histopathology

Gross pathology examination of dogs with DCM generally shows dilatation of either all four cardiac chambers or predominant dilatation of the left chambers. Myocardial eccentric hypertrophy is evident by increased heart

weight/body weight ratio, together with a decreased ratio of left ventricular wall thickness to chamber diameter.[10,15]

Histologic changes described in humans, dogs, and cats with idiopathic DCM include elongated, stretched or thinned out, so called attenuated or atrophied, often wavy, myofibers, fatty infiltration, vacuolization, hypertrophy of individual myocytes and variation of myofiber diameter, fibrosis, and rarely necrosis. Reports on histopathology in canine idiopathic DCM, excluding cardiomyopathy of boxers, are scant in breeds other than the Doberman pinscher (Table 1).

Author	Breed	Number of dogs	Histopathology
Tilley[25] (1975)	10 Gr. D., 1 Dob, 1 Ir.W.	12	attenuated wavy fibers
Van Fleet[124] (1981)	7 Gr. D., 1 Dob, 1 Ir. Set., 1 Lab., 1 St.B.	11	necrosis, hyperplasia of arteries, (6 dogs), fibrosis, vacuoles
Staaden[125] (1981)	English cocker spaniels	4	necrosis (3 dogs)
Gooding[126] (1982)	English cocker spaniels	1	focal necrosis
Calvert[27] (1982)	Doberman pinschers	6	fatty infiltration, fibrosis, atrophy
Hazlett[127] (1983)	Doberman pinschers	14	fatty infiltration, fibrosis, arteriosclerosis, degeneration of myofibrils
McCarthy[128] (1984)	2 Dob., 2 Ger. Sh., 2 Ir. W., 1 St. B.	7	focal necrosis and infarcts (2 dogs)
Sandusky[129] (1984)	2 Afg., 2 Dob., 3 Gr. D., 1 St.B.	8	attenuated wavy fibers, vacuoles, fibrosis, necrosis
Thomas[9] (1987)	English cocker spaniels	1	normal
Liu[130] (1989)	*	*	attenuated wavy fibers
Calvert[131] (1997)	Doberman pinschers	12	fatty infiltration, fibrosis, atrophy, hyperplasia of arteries
Dambach[21] (1999)	Portuguese water dogs	12	thin, wavy myofibers, i.e. attenuated wavy fibers, vacuoles
Everett[132] (1999)	Doberman pinschers	32	fatty infiltration, fibrosis, degeneration and atrophy of myofibers

Table 1. Studies reporting the histopathology of canine idiopathic DCM. (Afg=Afgan hound, Dob= Doberman pinschers, Ger.Sh=German shepherd, Gr.D.=Great Dane, Ir.set= Irish setter, Ir.W.=Irish Wolfhound, Lab.=Labrador retriever, St. B.=Saint Bernard), * information not available

The presence of attenuated wavy fibers is a major histologic finding in DCM in four reports in dogs,[21,25,129,130] and in two reports in humans.[103,133] Atrophy, or attenuation, of myofibers without a wavy appearance is described in an additional seven studies in humans.[5,134-139] Atrophy is a common response of muscle fibers to processes that prevent normal contractile activity, and to various pathologic stimuli.[140] Atrophy of cardiac myocytes has been shown to occur following prolonged mechanical support using left ventricular assist device systems,[141] and following heterotopic isotransplantation.[142] Myocardial atrophy has also been induced by hypothyroidism,[143] hypokinesia,[144] and malnutrition[145] in rats. Wavy myocardial fibers, especially when associated with focal edema, are characteristic signs of acute myocardial ischemia in humans,[146] and dogs,[146,147] but have also been described in human dilated cardiomyopathy.[148,149] On the contrary, hypertrophy of myocytes was reported in seven studies of human DCM, out of which five reported on concomitant myocyte atrophy or attenuation.[134-138,150,151] Whether the reported hypertrophy involved the entire myocyte or was confined to the nucleus is not clear in all studies.

Cardiomyopathy of boxers is histologically characterized by myocytolysis and myocyte degeneration, vacuolization, myocyte atrophy, and extensive fibrosis and fatty infiltration.[22,152] It has been compared to isolated right ventricular cardiomyopathy in dogs[153,154] and in humans.[155-158] Four different reports on histologic examinations of a total of 64 Doberman pinschers with DCM describe findings similar to cardiomyopathy of boxers, i.e. fibrosis, fatty infiltration, myofiber degeneration, myocyte atrophy, and sometimes vacuolization.[27,127,131,132]

In conclusion, the histopathologic characteristics of DCM in dogs, as presented in textbooks[10,15] and elsewhere refer to a limited number studies (n=13) describing a total of 120 dogs of which 58% (n=70) were Doberman pinschers (Table 1), with myocardial changes resembling the cardiomyopathy of boxers. Aside from the distinct histopathologic findings in the myocardium of Doberman pinschers and boxers, there appears another distinct histologic pattern displayed in a group of mainly large-breed dogs, i.e. that of attenuated wavy fibers.

5. Neuroendocrine response to congestive heart failure

Failure in cardiac function is met by compensatory responses in the cardiac, renal, central nervous and peripheral vascular systems. These systems operate under the influence of neurohormonal and endocrine factors such as RAAS, catecholamines, and vasopressin.[161] Although activation of these hormonal systems may be beneficial in preserving perfusion to vital organs, their effects are considered to be detrimental in a longer perspective, leading to fluid retention and increased peripheral vascular resistance, thus increasing the

work load of the failing heart.[159] It has been reported that the sympathetic nervous system is activated before the RAAS in dogs with pacing-induced heart failure.[106] It is well recognized that the RAAS promotes myocardial hypertrophy and fibrosis, i.e. excessive collagen synthesis, mainly through the action of AII and aldosterone, eventually leading to systolic and diastolic dysfunction.[160,161] Upregulation of the AII receptor gene associated with myocyte hypertrophy and fibrosis was reported in humans with DCM.[162] A myocardial RAAS with a local generation of AII has been reported by several authors,[163-165] and has been suggested to be the most significant pathway of the RAAS.[166] Myocardial stretch activates synthesis of AII and other RAAS components.[167,168] Activation of the circulating RAAS was reported in dogs with symptomatic DCM,[18] and in dogs with various heart diseases,[169] as well as in humans with DCM.[170-172]

In CHF, a counter-regulatory system of natriuretic peptide (ANP and BNP) production in both atrias and ventricles[173-175] inhibits the synthesis of renin and aldosterone, antagonizes the action of AII, decreases the activity of the sympathetic nervous system, and inhibits the release and action of vasopressin.[176,177] Atrial stretch,[175,178] increased atrial pressure,[179] endothelin, adrenergic stimulation,[175] and tachycardia[180] are major stimuli for ANP secretion. Atrial natriuretic peptide concentrations were increased 3-4 times in human patients with DCM, compared to patients with left-sided valvular heart disease.[181,182] A disturbed peripheral metabolism of ANP and a resistance to its biological effects have been demonstrated in human patients with DCM.[183] Plasma adenosine, another endogenous cardioprotective molecule, counteracting cathecholamine, renin-angiotensin, and cytokine induced cellular injury, was increased in human patients with heart failure.[184] Reports on activation of RAAS and ANP in dogs with asymptomatic heart disease, which may elucidate the need for therapeutic intervention in this stage of the disease, have so far been scant.[18,185]

6. Epidemiology, survival, and prognostic factors

The prevalence of myocardial disease, based on clinical and post mortem criteria, was estimated to be 1.4% in one study of 4831 dogs in 1965.[186] An Italian study of 7148 dogs estimated the prevalence of DCM at 1.1%.[187] A more recent survey of 342152 dogs registered in the Veterinary Medical Data Base at Purdue University found a prevalence of DCM at 0.5%.[10]

Prediction of survival times and identification of factors influencing mortality are of interest for both human and canine patients. Survival rates in human patients vary between 52 and 77% at one year and between 32 and 52% at two years, in different studies. Predictive factors of survival rate include clinical class of heart failure, increased left ventricular filling pressure, increased systemic vascular resistance, increased pulmonary capillary wedge pressure, left intraventricular conduction delay, increased hypertrophy-

dilatation index, Doppler indices, and Doppler-derived assessment of pulmonary hypertension.[188-193] Results are conflicting for the use of ventricular arrhythmia as a prognostic indicator.[195,196] In a study of 37 dogs with DCM, survival rate at one year was 37.5% and 28% at two years. The presence of pleural effusion and pulmonary edema were the only independent variables that significantly decreased survival.[194] Another study of survival and prognostic factors in 66 Doberman pinschers disclosed a mean and median survival time of 9.7 and 6.5 weeks, respectively, where shorter survival times were associated with atrial fibrillation and biventricular CHF.[31] Echocardiographic indices of left ventricular volume, but not FS or EPSS, proved to be prognostic markers in 31 dogs with DCM.[195]

AIMS OF THE THESIS

- to characterize the clinical, electrocardiographic, radiographic, echocardiographic, and pathologic findings in a homogeneous population of dogs (Newfoundlands, paper I) and in a large, and with respect to breed, heterogeneous population of dogs (paper II) with dilated cardiomyopathy.

- to estimate survival times and identify prognostic indicators for dogs presenting with congestive heart failure (NYHA class IV) attributable to dilated cardiomyopathy (paper III).

- to determine the prevalence of attenuated wavy fibers in the myocardium, and the sensitivity and specificity of this finding, of dogs with dilated cardiomyopathy (paper IV).

- to determine the sensitivity of standard clinical-echocardiographic criteria for DCM, using post mortem criteria for final diagnosis (paper IV).

- to determine the sequence of events regarding the development of attenuated wavy fibers in relation to echocardiographic evidence of chamber dilatation and myocardial hypokinesis in DCM (paper V).

- to investigate the activation of the renin-angiotensin-aldosterone system and atrial natriuretic peptide concentration in dogs with asymptomatic and symptomatic DCM (paper VI).

- to determine concentrations of circulating thyroid hormones in congestive heart failure due to dilated cardiomyopathy (papers I, II, VI), and whether the expression of mRNA coding for thyroid hormone receptor subtypes in the myocardium was altered in dogs with congestive heart failure (NYHA class IV) caused by DCM or, for comparative reasons, chronic valvular disease (paper VII).

MATERIALS AND METHODS

The materials and methods used in this thesis are presented in detail in the separate papers. This section contains general comments on the inclusion criteria, the dogs and the methodology.

1. Definitions of DCM and inclusion criteria

Dogs were considered to have **symptomatic** DCM if (1) fractional shortening, determined by means of M-mode echocardiography, was 25% or less[196], (2) echocardiographic lesions other than chamber dilatation were not evident during two-dimensional echocardiography, (3) radiographic or post mortem evidence of left-sided or biventricular enlargement in association with pulmonary edema or pleural effusion[197] (papers I-IV, VI), i.e. NYHA class IV heart failure. All three criteria had to be met by all dogs included in the studies. The presence of attenuated wavy fibers on histologic examination of the myocardium was added to the inclusion criteria for paper VII, based on the results of paper IV. **Asymptomatic,** or subclinical, DCM was diagnosed in dogs using echocardiographic criteria as above, in the absence of clinical and radiographic evidence of congestive heart failure[16-18] (paper VI). Newfoundlands, originating from a breeding kennel with a high prevalence of DCM, that were euthanatized for reasons unrelated to heart disease were used for studies of histopathology in paper V.

2. Dogs

A total of 230 dogs with dilated cardiomyopathy (208 dogs presenting in NYHA class IV congestive heart failure, 15 dogs with asymptomatic disease, and 7 dogs with only histopathologic evidence of DCM) were evaluated in the studies included in this thesis. For comparative reasons, 159 dogs with heart disease other than DCM (67 dogs with chronic valvular disease, 49 dogs with congenital heart disease, 26 dogs with myocardial infarcts, 9 dogs with myocarditis, 7 dogs with endocarditis, and one dog with cardiomyopathy of boxers), and 99 control dogs (37 age-, and sex-matched normal Newfoundlands, 8 Newfoundlands with non-cardiac diseases, 32 dogs who died suddenly or were euthanatized for reasons unrelated to heart disease, 15 age-, breed-, and sex-matched control dogs, and 7 research Beagles) were included. All dogs were privately owned, except for the 7 research Beagles who were euthanatized for other reasons. Permission to use myocardial specimens from the research dogs for the study of gene expression (paper VII) was granted by the Research Animals Ethics Committee in Stockholm. The number of dogs shared between studies of the included papers I-VII is presented in Table 2.

Paper	I	II	III	IV	V	VI	VII
I	-	33	33	19	0	0	0
	II	-	189	65	0	0	7
		III	-	65	0	0	7
			IV	-	0	0	0
				V	-	0	0
					VI	-	0
						VII	-

Table 2. Number of dogs shared by two studies included in the thesis.

All dogs in heart failure attributable to DCM were examined by the author at Albano Animal Hospital of Stockholm (92% of the dogs) or to one of five other animal hospitals in Sweden. Dogs with asymptomatic DCM were presented for reasons other than heart disease (paper VI). Age-, and sex-matched Newfoundlands (paper I) and Newfoundlands with occult DCM or with normal appearing myocardial specimens (paper V) participated in an ongoing survey of a breeding kennel with a high prevalence for DCM. Twelve of the dogs with heart disease other than DCM (papers IV, VII) were evaluated clinically and on post mortem examinations. All asymptomatic dogs, all Newfoundlands, and 9 of the 12 dogs with heart disease other than DCM which were evaluated clinically, were examined by the author. Only post mortem examinations were performed on the 147 dogs with heart disease other than DCM (paper IV), and on the 32 dogs who died or were euthanatized for reasons unrelated to heart disease (paper V). Age-, breed-, and sex-matched control dogs (paper VI) were recruited for each study, and examined by one of two cardiologists.
All but one of the DCM dogs in paper VII were treated with furosemide, five dogs with digoxin, and two dogs were treated with propranolol. In the CVD group all dogs received digoxin, furosemide, and enalapril.

3. Methods of examination

Electrocardiographic examination (papers I-IV, VI)
During ECG examinations, the dogs were placed unsedated in right lateral recumbency. Electrodes were attached, using alligator clips, to the skin proximal to the caudal aspect of the olecranon or over the patellar ligament, respectively. Alcohol was used as a conductive medium. Three channels were recorded simultaneously. A paper speed of 50 mm/s was used in all studies, and all measurements were obtained from lead II. Standard six-lead ECG were recorded and analyzed according to Tilley[198] by the author, and by an additional cardiologist in paper VI, who was blinded to the clinical diagnosis.

Radiographic examination (papers I-IV,VI)

Thoracic radiography in two orthogonal views was performed in all dogs (paper VI), and in left lateral recumbency in all clinically evaluated dogs (papers I-IV, VII). All radiographs were evaluated for heart size, signs of pulmonary congestion and edema, and pleural effusion,[197] by the author, and for paper VI also by a radiologist, who was blinded to the clinical diagnosis. Abdominal radiography to evaluate the presence of ascites was performed in a limited number of dogs (papers I and II).

Echocardiographic examination (papers I-VII)

M-mode and 2D echocardiography was performed using a 5 MHz transducer placed on the right precordium, with dogs (unsedated) positioned in right lateral recumbency. Echocardiograms were recorded and analyzed according to the recommendations of the American Society of Echocardiography[199] and the Echocardiographic Committee of the Speciality of Cardiology, American College of Veterinary Internal Medicine.[200] Ninety-two percent of DCM dogs in congestive heart failure, all asymptomatic dogs, all Newfoundlands, and 9 of the 12 dogs with heart disease other than DCM which were evaluated clinically, were examined with echocardiography by the author. The echocardiographic measurements of left atrial and left ventricular dimensions were corrected for the body weight in paper V and according to Kittleson[201] in paper VI. Previously published reference values were used.[202]

Post mortem examination and histologic evaluation (papers I-V, VII)

All post mortem examinations including the histologic evaluations were performed by a single pathologist. Histologic specimens were taken from the lower and the upper halves of the lateral walls of both ventricles; the proximal (1 to 1.5 cm distal to the base of the atrioventricular valves), distal (1 to 1.5 cm proximal to the apex), and middle (midway between the proximal and distal specimens) portions of the interventricular septum; and the papillary muscles of the left ventricle. Standard histologic techniques were used, including staining with H&E and Masson's trichrome stain. Three slides from each of the 9 specimens were examined. For paper IV the histologic examination was done sequentially, but at random, and the pathologist was blinded to the clinical diagnosis and gross pathologic findings at the time of histologic examination. For paper V additional specimens were taken from the middle portion of the ventricles from the Newfoundlands, and six specimens from the myocardium were examined from the non-Newfoundland dogs.

Attenuated wavy fibers were defined as myocardial cells < 6 μm in diameter (normal myofiber diameter ranges from 10 to 20 μm[203]) and with a wavy appearance. Dogs were considered to be positive for attenuated wavy fibers if at least half of the thickness of the specimen from the upper and lower

portions of the left ventricular wall were composed of attenuated wavy fibers.
Arteriosclerosis was defined as hyaline deposits in the arterial wall.[204] Chronic valvular disease was defined and classified (type 1 through 4) according to published criteria.[203] Cardiomyopathy of boxers was defined as left and right ventricular dilatation, atrophy and vacuolization of myofibers, fibrosis, fatty infiltration, and sometimes foci of mononuclear cell infiltrates.[152] Myocardial infarcts were defined according published criteria.[204] Myocarditis was defined as myofibers interspersed with inflammatory cells, hemorrhage and edematous fluid.[152] Endocarditis was defined as valvular or mural vegetations consisting of fibrin and aggregates of platelets, bacteria, and inflammatory cells.[205]

4. Assays

The TSH and TT4 assays were commercial tests marketed for use with canine samples. All other assays (NT-proANP, PRA, Aldosterone and FT4) were validated for canine plasma, but were not marketed primarily for use with canine samples. Thus for assays other than TSH and TT4 the relative changes observed in the measured parameter are accurate, but the absolute values obtained may not be, because the assays are heterologous for dog samples. Characteristics of the radio and chemiluminescent immuno assays used are summarized in Table 3.

Method	Type of assay	Extraction	LDL	Recovery (range)	CV Intra assay	CV Inter assay
NT-proANP[a]	RIA	No	62 pMol/L	91 to 108%	4 to 10%	9%
PRA[b]	RIA	Yes	0.13 ng/ml/h	NA	8%	<10%
Aldosterone[c]	RIA	No	32 pMol/L	71 to 98%	8%	10%
ACE activity[d]	REA	No	2 ACE units	NA	<1 to 8.2%	<1% to 2.9%
TSH[e]	CEIA	No	1.5 mIU/L	85 to 104%	3.9 to 10.8%	5.2 to 13.8%
TT4[f]	CEIA	No	1 nmol/L	96 to 110%	3.8 to 5.0%	6.3 to 8.2%
FT4[g]	CEIA	No	2.5 pmol/L	NM	5.3 to 8.1%	9.0 to 10.9%

Table 3. Abbreviations: CV-Coefficient of variation, LDL-Lowest detection level, NM-Not measured, NA-Not applicable, RIA-Radioimmunoassay, RE-radioenzymatic assay, CEIA- chemiluminescent enzyme immunometric assay.

[a] NT-ProANP (BT02), Biotop OY, Oulu, Finland, [b] GammaCoat™ PRA method, Incstar, Stillwater, MA, USA, [c] Coat-A-Count Aldosterone, DPC, Los Angeles CA, USA, [d] ACE direct-REA, Code RK-ACD, Bühlmann Laboratories AG, [e] IMMULITE Canine TSH, DPC, Los Angeles CA, USA, [f] IMMULITE Canine Total T4, DPC, Los Angeles CA, USA, [g] IMMULITE Free T4, DPC, Los Angeles CA, USA.

5. Methods to evaluate altered gene expression

Altered gene expression may be evaluated using a variety of approaches. One may choose between investigating the gene itself (DNA), the transcriptional product (mRNA), or the translational product (protein). There is however no complete concordance between transcription of a certain gene and translation of the corresponding protein. Secondly, there are different methods available for each of these investigations.

In paper VII we measured the mRNA for the thyroid hormone receptor, using a quantitative reverse transcription - polymerase chain reaction (RT-PCR) protocol.[206] The canine thyroid hormone receptors themselves are not suitable for analysis, as no subtype specific antibodies are available. As mRNA is very unstable due to the ubiquitous presence of RNA-degrading enzymes, it is converted via reverse transcription to the complementary DNA (cDNA).[207] PCR is an extremely sensitive and specific procedure to amplify the cDNA segment to a quantity sufficient for further analysis. Primers with a known base sequence are needed to initiate the PCR. As canine sequences were not known, human and murine primers were used. Amplification products were quantified by use of ELISA.

6. Statistical analyses

In papers I and II statistical significance was tested using two-tailed Student's t-test and chi-square analysis, as implemented in StatView512+ for Macintosh. Expected values calculated from a binomial distribution were used for comparison when $n<5$ (paper II). The minimum level of significance was chosen as $p<0.05$ (two-tailed).

In paper III statistical analysis was performed by a statistician using the statistical package SAS for Windows 6.10. (SAS Institute, Inc. Cary, N.C., USA) Survival curves were based on the Kaplan-Meier method. Survival curves of the discrete variables were compared using the Log rank test. Continuos variables were analyzed separately with the univariate Cox proportional hazard model. Variables that were statistically significant ($p<0.05$) were considered relevant for multivariate analysis, using the multivariate Cox proportional hazard model.

Statistical calculations in papers V and VI were performed by use of the statistical program JMP 3.2, SAS. Statistical methods used, in case of normally distributed data sets, were one-way analysis of variance (ANOVA), and the Tukey-Kramer test, which was applied for multiple comparisons. Equal variances between groups were tested using the F-test (variance ratio test). Logarithmic transformation was conducted to correct for non-normality. If different distributions between the groups could not be corrected by logarithmic transformation, non-parametric methods e.g. the Wilcoxon's rank sum test was applied. In cases of continuous variables, the Spearman's ρ

was used as a non-parametric measure of associations. Differences between groups with categorical data were determined by the chi-square test. Fisher's exact test was used when $n<5$. The minimum level of significance was chosen as $p<0.05$ (two-tailed).

In paper VII data were analyzed using ANOVA with a Fischer protected least significant test, as implemented in Statview v. 4.01, Abacus Concept Inc. Berkeley, CA, USA. Differences were considered significant at $p<0.05$.

RESULTS

1. Characterization of DCM in a homogeneous population of dogs (Newfoundlands), and in a large, and with respect to breed heterogeneous, population of dogs (papers I-II)

Breed, sex and age characteristics

Dilated cardiomyopathy was found in 189 dogs of 38 different, mainly medium-size and large, breeds with an over-representation of Airedale terriers, boxers, Doberman pinschers, English cocker spaniels, Newfoundlands, St. Bernards, and standard poodles, as compared to expected frequencies based on registration records of the Swedish Kennel Club. German Shepherd dogs were significantly under-represented. DCM was less common in giant breeds, except for Newfoundlands, and small breeds, with the exception of a papillon and three Cavalier King Charles spaniels. There was no statistically significant over-representation for either sex for the Newfoundlands (37 dogs), when compared to the sex distribution in a reference group of 155 clinically normal Newfoundlands, whereas there was a male preponderance in the heterogeneous group of dogs, when compared to registration records of the Swedish Kennel Club.

Age at onset of clinical signs varied from 3.5 months to 13 years, with a mean value of 5 years for the Newfoundlands and 6.6 years for the heterogeneous group of dogs. The proportion of dogs less than one year of age was remarkably high in the heterogeneous group of dogs, than in the patient population at the Albano Animal Hospital register during the same time period (Figure 1).

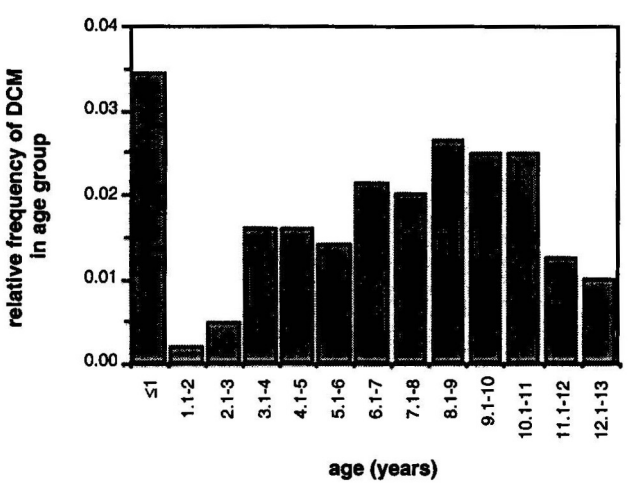

Figure 1. Age distribution of 189 dogs with dilated cardiomyopathy compared to age distribution of dogs visiting Albano Animal Hospital during 1987-1995.

Clinical findings

Presenting complaints included cough, depression, inappetence, dyspnea, weight loss, syncope, and polydipsia. Duration of clinical signs ranged from 0 to 240 days with a median of 7 days for both groups of dogs. Systolic murmurs were found in 11% in the Newfoundland group and in 25% in the heterogeneous group of dogs. Elevation of rectal temperature (>39°C) was noted in 52% of the Newfoundlands (n=21) and in 34% of the heterogeneous group of dogs (n=61). Polydipsia was reported in 16% and 19% of dogs in the heterogeneous and the Newfoundland group, respectively. Syncopal episodes were infrequent in dogs (17% in the heterogeneous group), although relatively more common in boxers (5/7) and in German Shepherd dogs (2/3). Hypercholesterolemia was present in 33% (n=58), and blood glucose was mildly elevated in 38% (n=65) of the dogs in the heterogeneous group.

Electrocardiography, radiography and echocardiography

Atrial fibrillation was the most common ECG abnormality, with an occurrence of 45% in both groups of dogs. Ventricular premature depolarizations (VPDs) were present in 16-18% of the dogs. VPDs were relatively more prevalent in boxers and in Airedale terriers (but not in Doberman pinschers) in this study.

Left-sided heart failure, as evidenced by pulmonary edema, was the most common radiographic finding (86% in the heterogeneous group and 91% in the Newfoundland group). Right-sided heart failure was less common (pleural effusion was seen in 11% of dogs in the heterogeneous group and in 18% of the dogs in the Newfoundland group). Ascites was a common finding (95% in the heterogeneous group and 100% the Newfoundland group) in dogs examined by abdominal radiography (n=21 and 5 in papers II and I, respectively).

Fractional shortening, determined by means of M-mode echocardiography, ranged from 5 to 22% (mean, 13%) in the Newfoundland group and from 5 to 25% (mean, 13%) in the heterogeneous group. Ten percent of healthy Newfoundlands in a sex,- and age-matched control group had FS <22% (paper I).

Post mortem findings

All four cardiac chambers were dilated in 85 and 100% of dogs in the heterogeneous group and the Newfoundland group, respectively. Left-sided heart failure (i.e. pulmonary edema) was evident in 85 and 95%, and right sided heart failure, i.e. pleural effusion (28 and 30%), hepatic congestion (67 and 75%) and ascites (30 and 40%) was present in the heterogeneous group (n=67) and the Newfoundland group (n=20) of dogs, respectively. Heart weight was recorded for nine of the Newfoundlands, ranging from 263 to 379 g (i.e. 193 to 292 g/m^2) with a mean of 328 g ($257 g/m^2$), which was a statistically significant difference from the normal value (120 g/m^2).

Histologic examination revealed a diffuse distribution of myocardial cells that were thinner than normal and had a wavy appearance, so called attenuated wavy fibers, in 89% of dogs in the Newfoundland group of dogs and 99% of the heterogeneous group of dogs. Diffuse, subendocardial interstitial fibrosis was found in 67% of the specimens. Concomitant myocardial infarcts were present in 13-16%.

2. Survival and prognostic factors in DCM (paper III)

Survival time ranged from 0 to 1640 days with a median of 27 days (mean 175 days). Ninety-two percent of the dogs died of cardiac-related causes. The majority of these dogs were euthanatized (74%). Thirteen dogs were alive by the end of the study, and three dogs were lost on follow-up. Survival rate at one year after initial diagnosis was 17.5% and 7.5% at two years.

Only three of 27 discrete and continuous variables, namely age at onset of clinical signs, dyspnea and ascites (as noted on physical examination), were of predictive value for the prognosis. Young age at onset of clinical signs was the most significant risk factor identified. Breed (including 44 Newfoundlands, 23 English Cocker spaniels, and 17 Doberman Pinschers), sex, and echocardiographic parameters (FS, EPSS, LA/AO, LWmean/LVEDD) were not correlated to the prognostic outcome of DCM in these 189 dogs. Neither were the presence of atrial fibrillation, ventricular premature depolarizations, or syncopal episodes correlated with survival times.

3. Sensitivity and specificity of the finding of attenuated wavy fibers in the myocardium of dogs with DCM, and the sensitivity of clinical-echocardiographic findings for the diagnosis of DCM in dogs (paper IV)

Attenuated wavy fibers were found in 64 of 65 dogs (representing 23 breeds, including 5 Doberman pinschers and 1 boxer) confirmed to have DCM on the basis of post mortem criteria, i.e. moderate to marked left-sided or biventricular dilatation and the absence of other cardiac abnormalities. Attenuated wavy fibers were most abundant subendocardially in the lateral wall of the left ventricle. Sixty-three percent of dogs also had signs of myocardial fibrosis. Cellular infiltrates were not detected. Attenuated wavy fibers were not found in the myocardium of 147 dogs with heart disease other than DCM, i.e. chronic valvular disease (n=60), congenital heart disease (49), myocardial infarcts (23), myocarditis (8), and endocarditis (7). Chamber dilatation was found in most dogs with chronic valvular disease (92%) or congenital heart disease (78%). Histologic findings of attenuated wavy fibers in the

myocardium of dogs were shown to have a sensitivity of 98% and a specificity of 100% for dogs with idiopathic DCM.
Sixty-five of 70 dogs (93%) with a clinical diagnosis of DCM, based on FS < 25% in the absence of abnormal echocardiographic findings other than chamber dilatation, and in the presence of radiographic or post mortem evidence of congestive heart failure, were correctly diagnosed, using post mortem criteria as above. The remaining 5 dogs had other heart diseases, such as arteriosclerosis, myocardial infarcts, endocardiosis, myocarditis, and cardiomyopathy of boxers, diagnosed on post mortem examination.

4. The sequence of events regarding the development of attenuated wavy fibers in relation to echocardiographic evidence of chamber dilatation and myocardial hypokinesis in DCM (paper V)

Attenuated wavy fibers were found extensively in the myocardium of 7 of 15 Newfoundlands (47%) from a kennel with a high prevalence for DCM (Table 4). All Newfoundlands were clinically normal and all measured echocardiographic variables were within the reference range for this breed (paper I). All dogs were euthanatized for reasons unrelated to heart disease. Median and mean ages of the dogs were lower than mean and median ages of Newfoundlands with clinical disease (paper I), and attenuated wavy fibers were even found in a 3-month-old puppy.
None of 32 dogs in a reference group of 23 different, non-Newfoundland breeds, who died suddenly (n=20) or were euthanatized (n=12) for reasons unrelated to heart disease, had attenuated wavy fibers on histologic examination of the heart.

Dog	Left ventricle				Right ventricle			Inter-ventricular septum		
	prox.	middle	distal	pap.m.	prox.	middle	distal	prox.	middle	distal
1	X	X	X	X			X	X		X
2	X		X	X	X	X	X	X		
3	X	X		X		X		X	X	
4	X	X	X	X	X	X	X	X	X	X
5	X	X	X	X				X	X	X
6	X	X	X	X	X	X	X	X		X
7	X	X		X	X		X			X

Table 4: Presence of attenuated wavy fibers in different parts of the myocardium in 7 Newfoundlands without clinical or echocardiographic evidence of heart disease. (See paper V for details)

5. Activity of RAAS and ANP in asymptomatic and symptomatic DCM (paper VI)

Plasma renin activity, PAC, urine aldosterone to creatinine ratio, and plasma NT-proANP concentrations were significantly elevated in symptomatic DCM dogs (n=15), as compared to both asymptomatic DCM dogs (n=15) and normal age-, breed-, and sex-matched control dogs (n=15). As expected, concentrations of PRA, PAC, urine aldosterone to creatinine ratio were significantly correlated to each other. Although plasma NT-proANP concentrations in asymptomatic DCM dogs were not significantly different from those found in normal dogs, the variation of NT-proANP values was greater than in the latter group. Plasma NT-proANP concentrations were particularly high in dogs with atrial fibrillation and appeared to increase with increasing heart rate, as well as with increased dimensions of the left atrium and ventricle (corrected for body weight according to Kittleson[201]), and with decreasing FS. ACE activity was comparable in all three groups.

6. Thyroid hormones in heart failure due to DCM or CVD (papers I,II, VI and VII)

Total thyroxine and T3 serum concentrations were not decreased in a majority of dogs with symptomatic DCM, as 82% of dogs (paper I, n=11) and 83% (paper II, n=58), respectively, were within the reference range. Serum concentrations of TSH and TT4 were within the reference range and did not differ significantly between the groups of symptomatic DCM dogs, asymptomatic DCM dogs, and normal control dogs. However, concentrations of FT4 were significantly decreased in dogs with symptomatic DCM, as compared to concentrations in asymptomatic dogs and normal control dogs (paper VI).

At the transcriptional level for the thyroid hormone receptor, concentrations of mRNA for receptor subtypes ß1 and ß2 were significantly increased (three fold and eight fold, respectively) in the myocardium of dogs with congestive heart failure attributable to DCM or CVD, compared to normal control dogs. There was no statistically significant difference in TR ß1 and ß2 mRNA upregulation between dogs with DCM and CVD. Thyroid hormone receptor α1 could not be detected using primers based on human or murine sequences. There was no difference in the concentration of the non-ligand binding TR α2 mRNA between dogs with DCM and CVD and normal control dogs. The ratio of expression of TR α2, TR ß1, and TR ß2 was 100:10:1 (paper VII). Homology between canine, murine, and human DNA sequences and the amino acid sequences for the thyroid hormone receptors α2, ß1, and ß2 is shown in Figure 2.

X07751 1155-1342 (mouse) vs. AF110378 (dog) α2-receptor

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CGGCCGGGCGGGTCACTGGGCGTCCACCCGGAAGGACAGCAGCTTCTCGGAATGCATGTT
************************************************************
CGGCCGGGCGGGTCACTGGGCGTCCACCCGGAAGGACAGCAGCTTCTCGGAATGCATGTT
 R  P  G  G  S  L  G  V  H  P  E  G  Q  Q  L  L  G  M  H  V

GTTCAGGGTCCGCAGGTCCGGCAGCTTGAGCAGCAGCTTGGTGAAGCGGGAAGTCTCCGA
************************************************************
GTTCAGGGTCCGCAGGTCCGGCAGCTTGAGCAGCAGCTTGGTGAAGCGGGAAGTCTCCGA
 V  Q  G  P  Q  V  R  Q  L  E  Q  Q  L  G  E  A  G  S  L  R

GGGCCGGTTCTTCAGCACCAGAGCCCGAAGAGCCCGCAGCAGCGTCTCCTGGAGCTGCTC
********************** ***********************************
GGGCCGGTTCTTCAGCACCAGAACCCGAAGAGCCCGCAGCAGCGTCTCCTGGAGCTGCTC
 G  P  V  L  Q  H  Q  N  P  K  S  P  Q  Q  R  L  L  E  L  L
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X04707 316-499 (human) vs. AF110379 (dog) ß1-receptor

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CTTACAGCTTGGGACAAACCGAAGCACTGTCCAGACCGAGAACACGACTGGAAGCTAGTA
************************************ **********************
CTTACAGCTTGGGACAAACCGAAGCACTGTCCAGACCAAGAACAAGACTGGAAGCTAGTA
 L  T  A  W  D  K  P  K  H  C  P  D  Q  E  Q  D  W  K  L  V

GGAATGTCTGAAGCCTGCCTACATAGGAAGAGCCATTCAGAGAGGCGCAGCACGTTGAAA
******************************** **** * ********************
GGAATGTCTGAAGCCTGCCTACATAGGAAGAACCATGCGGAGAGGCGTAGCACGTTGAAA
 G  M  S  E  A  C  L  H  R  K  N  H  A  E  R  R  S  T  L  K

AATGAACAGTCGTCGCCACATCTCATCCAGACCACTTGGACTAGCTCAATATTCCATCT
************** *********** *** ********** ***********
AATGAACAGTCGTCACCACATCTCATTCAGGCCACTTGGACTAGCTCGATATTCCATCT
 N  E  Q  S  S  P  H  L  I  Q  A  T  W  T  S  S  I  F  H
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X74497 233-365 (human) vs. AF110380 (dog) ß2-receptor

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CTGTGCCCAGTAACAACATATATATGGAACAGGCCTGGGCAGTGAATCAGCCTTATACC
******* * *  **************  ** ***** ************************
CTGTGCCAATTTTCAACATATATATGCAATTGGCCAGGGCAGTGAATCAGCCTTATACC
   V  P  I  F  N  I  Y  M  Q  L  A  R  A  V  N  Q  P  Y  T
TGTAGTTACCCTGGAAACATGTTTAAAAGCAAGGACTCTGACTTGGACATGGCCCTGAA
********************** * ***********************************
TGTAGTTACCCTGGAAACATGCTGAAAAGCAAGGACTCTGACTTGGACATGGCCCTGAA
 C  S  Y  P  G  N  M  L  K  S  K  D  S  D  L  D  M  A  L  N
TCAATACAGCCAACC
***************
TCAATACAGCCAACC
  Q  Y  S  Q
```

Figure 2. DNA sequence and corresponding amino acid sequence of thyroid hormone receptors α2, ß1and ß2. The upper sequences are of murine or human origin, and the lower sequences are of canine origin. * siginifies identical nucleotides.

GENERAL DISCUSSION

1. Histopatholgy, etiology, and classification of canine idiopathic dilated cardiomyopathies

There appears in the literature and in the present thesis to be two histologically distinct forms of canine idiopathic dilated cardiomyopathy. The cardiomyopathy of boxers[22] and of Doberman pinschers,[27,127,131,132] corresponding to the "fatty infiltration-degenerative" type, or "myofiber degeneration and atrophy, and replacement by collagen and adipocytes" classification used by Everett *et al.*,[132] and the form seen in many large- and medium-sized breeds (including some boxers and Doberman pinschers), which can be classified as the "attenuated, wavy fiber" type as described in papers I, II, IV,V,VII and by others (Table 5).[21,25,129,130] The classification into fatty infiltration-degenerative type and attenuated wavy fiber type of cardiomyopathy in dogs is superior to classification suggesting breed-specific syndromes, as some breeds (i.e. Doberman pinschers and boxers) may be affected by both diseases (paper IV).[25,129] The myocardial lesions associated with the attenuated wavy fiber type of DCM have a very high sensitivity (98%) and specificity (100%) for DCM (paper IV). The attenuated wavy fiber type of DCM may affect many breeds, and no differences between the breeds concerning histopathologic characteristics were found (papers I, II, IV).

Attenuated wavy fiber type **Fatty infiltration-degenerative type**

Author	Breed	Number of dogs in study	Author	Breed	Number of dogs in study
Sandusky[129]	Large and giant breeds	11	Harpster[22]	Boxers	64
Tilley[25]	Large and giant breeds	12	Hazlett[127]	Doberman pinschers	14
Dambach[21]	Portuguese water dogs	12	Calvert[27]	Doberman pinschers	6
Tidholm (papers I-IV, VII)	23 large and medium-sized breeds	64	Calvert[131]	Doberman pinschers	12
Tidholm (paper V)	Newfoundlands	7	Everett[132]	Doberman pinschers	32
Tidholm (unpublished data)	Newfoundlands + 5 other breeds	33			
Total number of dogs		139			128

Table 5. Classification of canine idiopathic dilated cardiomyopathies in different studies, based on the reported histopathologic characteristics.

Although there seems to be some differences between the two diseases in clinical, electrocardiographic, and prognostic respects,[8,15,31] the definite diagnosis will rest on histopathology, and may most likely only be made post mortem, as endomyocardial biopsies collected in vivo from the left ventricle is technically difficult, and biopsies from the right ventricle may not be sufficient for diagnosis (papers IV, V). Using the histologic characteristics in the myocardium of dogs with dilated cardiomyopathy to classify the type of cardiomyopathy may also facilitate the differentiation of the clinical, electrocardiographic, echocardiographic, and prognostic characteristics in each of the two types of diseases, thus aiding the ante-mortem diagnosis. The sensitivity of the standard clinical-echocardiographic diagnosis of symptomatic DCM is 93% in this study (paper IV). The diagnostic accuracy in differentiating idiopathic, or primary, cardiomyopathies and secondary cardiomyopathies, may be enhanced using histopathologic examination of the myocardium. Differentiating between different types of cardiomyopathies may not be of major clinical importance, but is of great interest in the research setting.

The development of attenuated wavy fibers may precede cardiac dilatation and hypokinesis as is shown in paper V, and may therefore be considered an early lesion of DCM, which may be denoted "occult DCM". As attenuated wavy fibers were not found in dogs with cardiac dilatation caused by heart diseases other than DCM (paper IV), this myocyte abnormality does not seem to be inducible by chronic volume overload and stretching of the myocytes, as has been suggested.[133] In humans with end-stage dilated cardiomyopathy, development of attenuated wavy fibers seems to be reversible with unloading of the heart,[133,148] which may implicate mechanical involvement in the pathogenesis of attenuated wavy fibers.

Presumably, specific histologic myocardial changes reflect specific disease processes. One can only speculate upon what disease process may be causing the lesions in the attenuated wavy fiber type of DCM. As the very structure of the myofibers is distorted, the cause may be a defect in the cytoskeletal proteins, such as dystrophin[41-44], actin[46,47] metavinculin, α-dystroglycan, α- and γ-sarcoglycan, and muscle LIM protein.[45] The lack of abnormalities found in the actin gene in Doberman pinschers,[47] presumably affected with the fatty infiltration-degenerative type of DCM, does not preclude its involvement in the pathogenesis of the attenuated wavy fiber type of DCM, as such involvement has been shown in human DCM.[46] It has been suggested that alterations of sarcomeric proteins, responsible for force generation, will lead to HCM, whereas alterations of cytoskeletal proteins, mainly involved in force transmission, will lead to DCM.[208] Other possible mechanisms involve oxygen delivery or energy-dependent processes,[117-119] as a wavy appearance of the myocyte has been associated with myocardial ischemia in humans and dogs.[146,147]

The attenuated wavy fiber type,[103,133] and attenuation[5,134,136,137,139,209] and waviness[148,149] of myofibers have been described in humans, as well as the fatty infiltration type[210] of mainly the right ventricle in arrhythmogenic right ventricular cardiomyopathy.[155-158] Unspecific findings, such as necrosis, found in a few studies[125,126,128,129] in a limited number of dogs (n=12), may be caused by various cardiac insults and toxins. Replacement of the myocytes with fibrotic tissue is reported as an unspecific finding in several studies of DCM in both humans and dogs.[5,27,102,124,127,131,135,137,138,150,151] Fibrosis, which often accompany attenuated wavy fibers (papers I, II, IV), has been shown to be influenced by RAAS activation, mainly AII and aldosterone, as a general response to myocardial failure. Vacuolization of myofibers is infrequently detected in the attenuated wavy fiber type of DCM (Lennart Jönsson, unpublished data), but is more often reported in the fatty infiltration-degenerative type of DCM,[22,152] and in DCM of the Syrian hamster.[211] Intracellular vacuolization may be caused by distention of the sarcoplasmatic reticulum and T-tubules, as in doxorubicin induced cardiomyopathy.[102] Myocyte hypertrophy, often described in human DCM,[134,135,138,150,151] seems to be lacking in studies of canine DCM. Evidence of eccentric hypertrophy, where sarcomerogenesis occurs in series rather than parallel, is present and manifest as increased heart weight in dogs with DCM (paper I). Beneficial and detrimental effects of myocardial hypertrophy are debated.[212,213] Myocardial hypertrophy, which is stimulated by increased wall stress, and will in turn decrease wall stress, was correlated with increased survival in human DCM,[191,213] but not in dogs in this study (paper III). The presence of attenuated wavy fibers may prevent adequate compensatory myocyte hypertrophy in the myocardium of dogs with DCM.

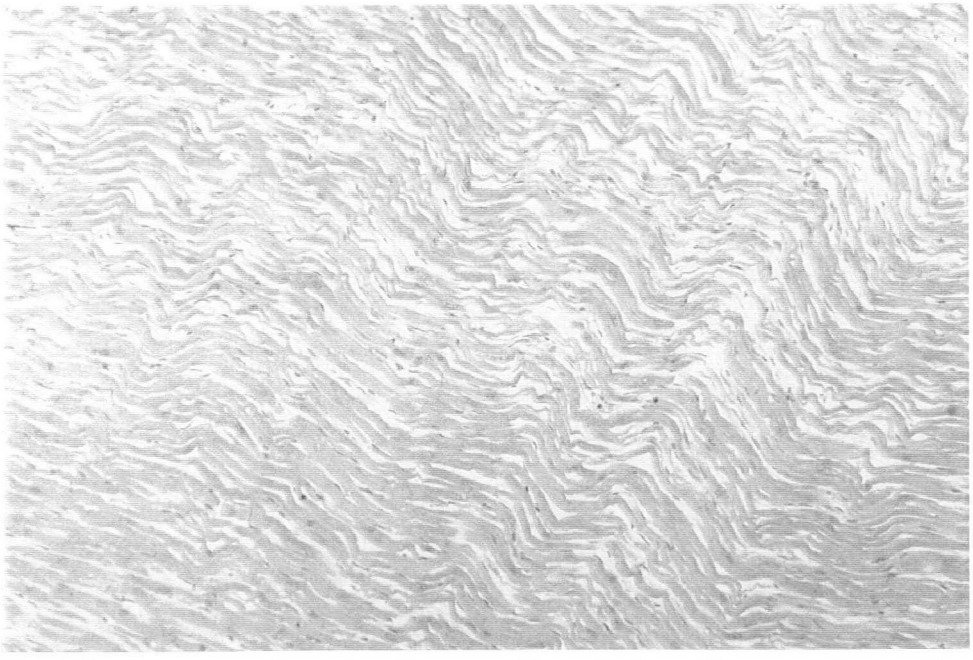

Figure 3. Histology specimen from the myocardium of a dog with attenuated wavy fiber type of canine idiopathic DCM. The myocytes are thinner than normal and have a wavy appearance. The myocytes are separated by a clear space, indicating edematous fluid, that is generally free from cellular infiltrates. H&E stain.

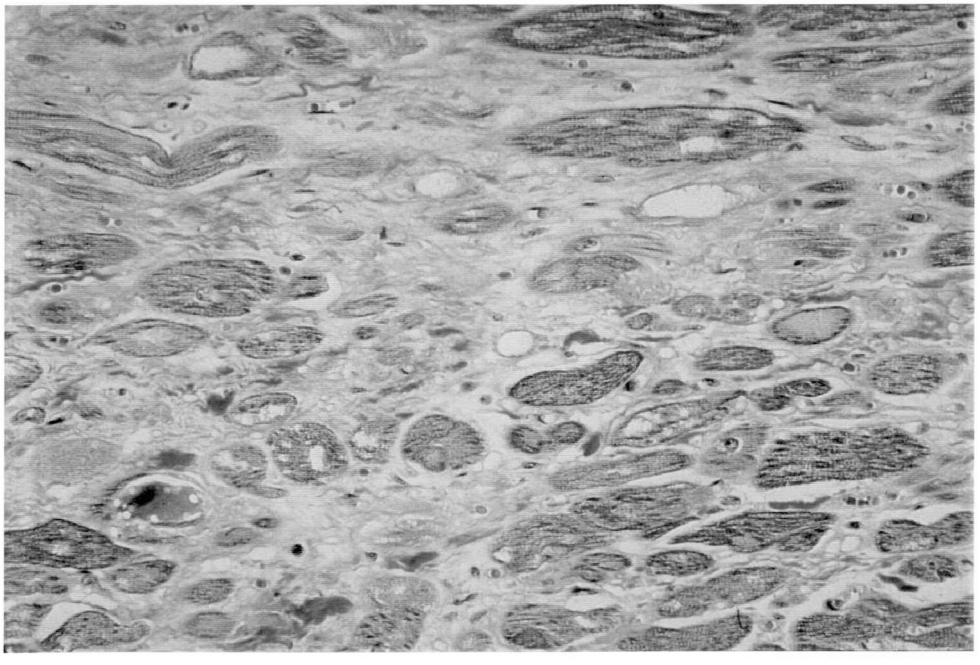

Figure 4. Histology specimen from the myocardium of a dog with fatty infiltration-degenerative type of canine idiopathic DCM. Vacuolization and fragmentation of myocytes as well as prominent proliferation of connective tissue are evident. Trichrome stain.

2. Clinical, electrocardiographic, radiographic, and echocardiographic characteristics in dogs with congestive heart failure caused by DCM

Until recently, previous characterization of canine DCM was based on relatively small numbers of dogs (8-23 dogs in each of several different reports),[12,26,125,214] and different inclusion criteria have been used. A large study, based on a search of the Veterinary Medical Data Base (VMDB) at Purdue University, included 1681 dogs with DCM. These dogs were coded as having acquired, congestive, or right-sided cardiomyopathy, but inclusion criteria were not further defined, and no reference to the clinical stage of the disease was made.[10] Fourteen breeds were represented in the study, and the most commonly affected, displayed as percentages of new hospital admissions, were Scottish deerhound, Doberman pinschers, Irish wolfhounds, Great Danes, boxers, St. Bernards, Afghan hounds, and Newfoundlands. In paper II, reporting of a heterogeneous population of 189 dogs of 38 different breeds, diagnosis of congestive heart failure caused by DCM was based on clinical, radiographic or post mortem, and echocardiographic criteria. Seven breeds were significantly over-represented compared with expected frequencies based on registration records of the Swedish Kennel Club, namely Airedale terriers, boxers, Doberman pinschers, English cocker spaniels, Newfoundlands, St. Bernards, and standard poodles (paper II). The prevalence of DCM increased with age in the VMDB survey. In paper II, the number of dogs less than one year of age was remarkably high compared to the age distribution of dogs admitted to Albano Animal Hospital. Few dogs developed signs of the disease between the ages of one to two years. The number of dogs with DCM increased between three and nine years of age, after which it decreased. A male predominance was found in paper II as well as in the VMDB survey and other studies.[12,19,26-28,31] It should be noted that the inherent weakness of any case series study, is that the sample may not be representative of the population. The large number of possible biases in observational studies can lead to considerable variation in the findings from similar studies.[215]

Polydipsia, seldom reported in association with DCM, was found in approximately one fifth of the dogs, was most likely due to stimulation of the thirst-vasopressin system, possibly mediated through increased RAAS activity,[216,217] as shown in paper VI. Echocardiographic findings were in agreement with previous studies,[12,13,15] and FS of 22% or less, as shown in paper I, may be a useful criteria for clinical diagnosis of DCM in Newfoundlands. Breed specific echocardiographic variables have been reported,[218-222] but it should be emphasized that reference values for echocardiographic variables for all the different breeds presented in papers II - IV and VI were not available.

When comparing the group of Newfoundlands (paper I) with expected values calculated from a binomial distribution for the breed heterogeneous group of

dogs (paper II) with DCM, no statistically significant differences were found, with the exception of a higher frequency of four chamber dilatation on post mortem examination, a lower frequency of systolic heart murmurs, and an increased prevalence of elevated body temperature in the Newfoundland group. There was no statistically significant sex predisposition in the Newfoundland group as opposed to the group of breed heterogeneous group. The reference groups used for estimation of sex-predilection were different in papers I and II, which may influence the results. Right-sided heart failure seems to be common in giant breeds with DCM.[223] The systolic murmurs associated with DCM are usually of low intensity, and may be difficult to auscultate, especially in the Newfoundland breed, where panting is common. Systolic murmurs in the heterogeneous group of dogs were also less frequent than previously reported.[13,15,26] Severe heart failure is often accompanied by fever, possibly associated with increased levels of tumor necrosis factor.[224,225] Also, peripheral vasoconstriction, induced by activation of the neuroendocrine system, may decrease loss of body heat. It may be that elevations of body temperatures are greater in Newfoundlands compared to other breeds, because their thick, often black, haircoat prevents the effective escape of body heat. In accordance with previous reports,[15,26] left-sided heart failure, was the most common radiographic finding. Atrial fibrillation was the most common arrhythmia in both groups (45-46%), which is similar to that reported in other studies,[25,26] except for studies of boxers and Doberman pinschers (11-18%).[28,152] Compared to studies of the latter breeds, where reports of ventricular dysrhythmias are frequent (46-92%), the prevalence of VPDs was low (16-18%) in our studies, although relatively more common in Airedale terriers and boxers, but not in Doberman pinschers. The explanation for this discrepancy may stem from the fact that the boxers and Doberman pinschers in those studies were afflicted with the fatty infiltration-degenerative type of DCM, and that the dogs in our studies predominantly were diagnosed as the attenuated wavy fiber type of DCM.

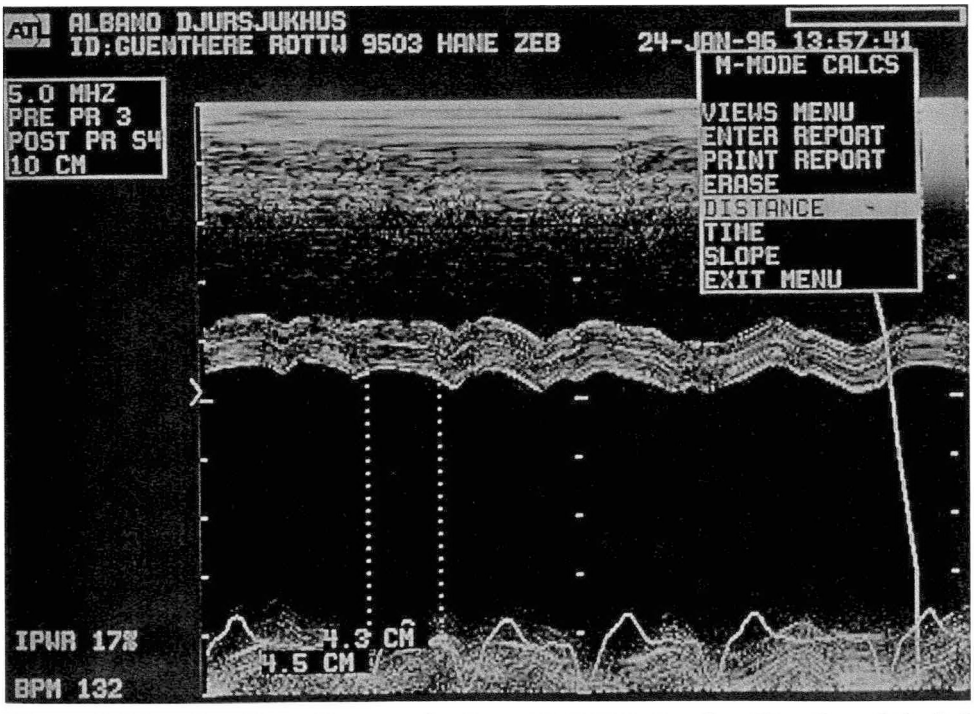

Figure 5. M-mode echocardiogram of a dog with DCM, showing dilatation of the left and right ventricles and severe left ventricular hypokinesia.

3. Survival and prognostic indicators in DCM

Survival analysis of 189 dogs in NYHA class IV congestive heart failure of this study (paper III) reveals a poor prognosis with survival rates of 17.5% at one year and 7.5% at two years after onset of clinical signs. Survival times ranged from 0 to 1640 days, with a median of 27 days, (mean, 175 days). Survival times are compared with other studies in dogs in Table 6. Survival rate at one year was exceptionally low in the study of Doberman pinschers,[31] which may be an indication of difference in prognosis between the fatty infiltration-degenerative type and the attenuated wavy fiber type of DCM. Medical treatment, especially ACE inhibitors[226-229] and ß-blockers,[230] is known to influence survival in dogs and humans with CHF, but was only evaluated in two of the studies. However, as the administration of ACE inhibitors and ß-blockers varied considerably, this may also be a source of variation of survival times between the studies (Table 6).

	Paper III	Monnet et al.[194]	Calvert et al.[31]	Borgarellli et al.[195]	Ettinger et al.[227]	Bench study[226]
Number of dogs	189 (38 breeds)	37 (12 Dob)	66 Dob	31 (11 Great Danes)	43 (24 Dob)	37
Functional class of heart failure	IV*	I-II*: 13 dogs III-IV*: 24 dogs	IV*	IV***	III*or IV*	III** or IV***
Median survival time	27 days	69 days	45.5 days	120 days	50 days (placebo) 130 days (enalapril)	250 days (placebo) 100 days (benazepril)
Survival rate at one year	17.5%	37.5%	3%	44%	-	22% (placebo) 46% (benazepril)
Survival rate at two years	7.5%	28%	-	-	-	-
Percentage of dogs given ACE inhibitors	9%	27%	38%	100%	49%	46%
Percentage of dogs given ß-blockers	22%	11%	7.6%	-	-	-

Table 6. Comparisons between studies of survival in dogs with DCM. (Dob= Doberman pinschers) * NYHA class, ** =ISACHC[231] class II, *** = ISACHC class III

Medical treatment of congestive heart failure may include inotropic support, diuretics, and inhibitors of the RAAS and/or the sympathetic nervous system. Increase in myocardial contractility may be accomplished either by direct changes in ion influx (digoxin) or by increase of cAMP, either by ß-adrenergic stimulation (Dobutamin), or via inhibition of phosphodiesteras (amrinone, milrinone, enoximone). Several inotropic agents, such as pimobendan and vesnarinone, have additional modes of action.[232,233] Increased survival was found in dogs with DCM that responded with increased FS when given digoxin, compared to dogs that did not respond.[234] Improvement of cardiac function[235,236] and clinical signs,[237,238] but not of survival times,[237] has been shown in human patients with congestive heart failure treated with digoxin.[232] Increased mortality was reported in a human study involving milrinone, and conflicting results concerning the efficacy of phosphodiesteras inhibitors have been reported in both humans and dogs.[239-241] Pimobendan, a phosphodiesteras inhibitor with calcium-sensitizing properties, was shown to increase survival time in Doberman pinschers, but not in English cocker spaniels, with DCM,[242] and clinical improvement with its use has been reported for dogs[243-245] and humans.[246] Heart failure may be viewed, not only as a hemodynamic illness, but as an illness of abnormal growth and remodeling of the heart, a process which may be attenuated or reversed via ACE inhibition and ß-adrenergic blockade.[146] Reports on beneficial effects of ACE inhibition are numerous in both humans[228,229] and dogs.[226,227,247,248] However, no studies have shown statistically significant increased survival times for dogs with DCM.[226,227] The use of ß-adrenergic blocking agents in human DCM was advocated in 1975 by Waagstein *et al.*, who used alprenolol or practolol and showed improved left ventricular function.[249] Beneficial effects have also been reported in studies of the ß1-selective blockers metoprolol[250-252] and bisoprolol,[253] and the mildly selective ß1-blocker and α1-blocker carvedilol.[254] A meta-analysis of 35 reports of ß-blockade administration showed reduced mortality in human patients in CHF.[230]

Prognostic indicators at time of diagnosis would be valuable information for owners and for veterinarians treating dogs with DCM. However, paper III shows that the prognosis in the individual case of DCM is difficult to predict at presentation, as only three of 27 discrete and continuous variables were shown to influence survival. Interestingly, young age at onset of clinical signs was the most significant risk factor identified. One can only speculate on the causes of this finding as to whether DCM may be a multifactorial, polygenic disease where in some dogs several genes are afflicted, which induces clinical disease at an early age and carries a worse prognosis. As expected, dyspnea and ascites (as identified on physical examination), i.e. signs of severe CHF, also correlated with increased mortality. It is noteworthy that breed did not influence survival, although 44 Newfoundlands, 23 English cocker spaniels, and 17 Doberman pinschers were included in the study. DCM in these breeds often are referred to as carrying different prognoses, especially the poor prognosis of the Doberman pinscher.[15,26,31] These contradictory findings

may be due to the fact that the dogs presented in this study are largely affected by the attenuated wavy fiber type of DCM, and previously studied Doberman pinschers may predominantly be affected by the fatty infiltration-degenerative type, which seems to carry a worse prognosis than the former type. Atrial fibrillation did not influence survival in this study, contrary to results in a study of 66 Doberman pinschers.[31] Nor did the presence of VPCs predict prognosis in our study, as identified by Monnet,[194] or echocardiographic parameters, as shown by Borgarelli.[195] Limitations of survival analyses of these kinds are discussed in paper III.

4. Neuroendocrine activation in asymptomatic and symptomatic DCM

Results presented in paper VI show that the neuroendocrine systems are activated in dogs with symptomatic DCM, as elevated concentrations of PRA, PAC, and urinary aldosterone to creatinine ratio were found, but not in dogs with asymptomatic DCM, when compared to age-, breed-, and sex-matched control dogs. Renin release is stimulated by reduced extracellular fluid volume, reduced systemic blood pressure, and increased sympathetic output.[255,256] Activation of the RAAS has been previously demonstrated in dogs with congestive heart failure caused by DCM[18]and by various cardiac diseases.[169] The lack of activation of systemic RAAS in dogs with asymptomatic disease in this study (paper VI), i.e. having echocardiographic evidence of left ventricular dilatation and hypokinesis in the absence of clinical signs of heart failure,[16-18] does not preclude the possible activation of the myocardial RAAS, where local generation of AII has been reported by several authors.[163-165] The RAAS activation in congestive heart failure, although possibly initially beneficial, is considered to be detrimental in a longer perspective, leading to fluid retention, increased peripheral resistance, and myocardial hypertrophy and fibrosis.[159-161] Beneficial effects of ACE inhibition in symptomatic heart disease is well documented.[226-229,247,248] Therapeutic decisions on whether to treat dogs with asymptomatic DCM with ACE inhibition should preferably rest on controlled clinical trials rather than the analysis of systemic RAAS activation.

Counter regulatory, "cardioprotective", hormonal systems include the endogenous production of natriuretic peptides,[173,174] and adenosine.[184] Atrial natriuretic peptide inhibits the synthesis of renin and aldosterone, antagonizes the action of AII, decreases the activity of the sympathetic nervous system, and inhibits the release and action of vasopressin.[177,180] Elevated concentrations of plasma NT-proANP, which is released on an equimolar basis with the C-terminal active hormone, but more stable in vitro,[257] were found in dogs with symptomatic DCM, but not in dogs with asymptomatic DCM, as compared to age-, breed-, and sex-matched control dogs. Increased concentrations of NT-proANP were significantly correlated with increased

heart rate, increased echocardiographic dimensions of the left atrium and ventricle, and decreased FS, results which in are in general similar to those in previous studies in dogs,[185,258] and human patients with CHF.[174,175,178-180] Activation of ANP appears to be more pronounced in DCM compared to left-sided valvular heart diseases in human patients.[181] Expression of ANP seems to be positively related to decreased survival in patients whit CHF.[259] Thus, the plasma concentrations of ANP may potentially be useful as a prognostic marker in canine patients with DCM. A disturbed peripheral metabolism of ANP and a resistance to its biological effects have been demonstrated in human patients with symptomatic DCM, as well as in those with asymptomatic DCM, who have ANP circulating levels and atrial pressure and volume within normal ranges.[183] These findings provide an explanation of the mechanism for the proposed benefit of ANP to patients with CHF.[260,261]

5. Involvement of the thyroid hormonal system in heart failure

The action of thyroid hormones has a great impact on cardiac performance.[61,63,262,263] The biologically active hormone T3 binds to thyroid hormone receptors in the myocyte nucleus, thereby inducing transcriptional activity on target genes coding for proteins involved in cardiac function, such as Na,K-ATPase, Ca-ATPase, heavy myosin chains, and ANP.[57,58,264,265] The enzyme Na,K-ATPase decreases intracellular sodium in exchange for potassium, thus improving myocardial relaxation. The Ca-ATPase will also enhance diastolic function by increasing reuptake of calcium ions into the sarcoplasmatic reticulum. Triiodothyronine increases the expression of MHC α-isoform at the expense of the ß-isoform, thereby increasing myocardial contractility. The synthesis of ANP is also increased under the influence of T3. Thyroid hormone increases myocardial hypertrophy by increasing total amount of contractile protein, primarily α-myosin and α-actin.[266] Triiodothyronine can also alter the relationship between the sympathetic nervous system the cardiovascular system by increasing sympathetic activity or enhancing responsiveness of the myocardium to adrenergic stimuli by increasing the number of ß-adrenergic receptors.[267]

It has been suggested that hypothyroidism may play a role in the development of DCM in dogs, although most studies fail to identify any such consistent relationship.[27,67] In this thesis (papers I, II, VI) no convincing evidence of low concentrations of TT4, T3 or high concentrations of TSH, as evidence of thyroid hormonal disturbance, were detected. The fact that FT4 , but not TSH or TT4, was significantly decreased in dogs with symptomatic DCM, in comparison with dogs with asymptomatic disease or normal control dogs in paper VI, may be due to increased protein-binding of T4, or to increased conversion of FT4 to T3. Concentrations of FT4, although only 1/1000 of the TT4 concentrations and thus more difficult to measure correctly, are

sometimes regarded as being more accurate than TT4 in detecting hypothyroid states. In general a TSH stimulation test is needed to verify the diagnosis, if results of baseline TT4, FT4 or TSH are discordant.[268] However, given that serum concentrations of TT4 and FT4 often decrease in dogs as a result of concurrent illness, i.e. "sick euthyroid syndrome",[269] such as congestive heart failure, and the fact that serum concentrations of the biologically active hormone T3 does not accurately reflect thyroid hormonal status in the dog,[268] the value of determining serum concentrations of thyroid hormones in dogs with systemic disease must be limited in this species.

The adaptation of the cardiomyocyte to pathologic conditions such as heart failure entails qualitative and quantitative alterations in gene expression. Gene products, i.e. proteins, include hormone receptors, which when activated, may induce profound effects on cell function. Downregulation or upregulation of hormone receptors may be of interest for therapeutic interventions in different disease states. In paper VII, mRNA for the T3 receptor subtypes α1, α2, ß1, and ß2 was studied, in order to investigate whether levels of T3 receptor subtypes are changed in the myocardium of dogs with CHF caused by DCM. To evaluate whether possible differences were inherent to DCM, or secondary to CHF irrespective of cause, dogs with CVD were included in the study. Concentrations of mRNA coding for TR subtype α1 could not be determined in the analysis, using murine and human primers in the RT-PCR technique used for detection and quantification. Tissue concentrations of mRNA for the non-ligand binding TR subtype α2 did not differ between dogs in CHF and normal control dogs, but was the most abundant receptor isoform. The role of the non-ligand binding receptor is uncertain as it does not show any sign of hormone related activity,[270] but may have a regulatory function on the ligand-binding subtypes. Messenger RNA, coding for TR subtypes ß1 and ß2, were upregulated threefold and eightfold, respectively, compared to control dogs, and to an equal extent in dogs with CHF attributable to DCM or CVD. This suggests that upregulation of mRNA for TR subtypes is not part of a specific disease process, but rather a reactive myocardial response to the increased hemodynamic stress imposed by CHF. Propranolol, known to decrease peripheral conversion of T4 to T3[271] and to cause downregulation of TRα1 and TRß1 in mice,[272] was administered to only two of the DCM dogs in paper VI and to none of the CVD dogs, and is thus unlikely to have influenced the results of the study. The difference in age between dogs with DCM and dogs with CVD was inherent to the different diseases. There was, however, a greater difference in age between clinically normal dogs and dogs with CHF. Most studies in dogs indicate that there is a slight decrease in circulating TT4 with increasing age, most likely caused by decreased production, decreased binding to plasma proteins, increased clearance, or increased receptor number or affinity.[268] Expression of TRß1 and ß2 mRNA is stable with age in humans.[273] Thus, results of paper VII suggest that the threefold to eightfold increase of mRNA coding for TR subtypes ß1 and ß2 is attributable to congestive heart failure in dogs, and not to differences in age.

The direct cause of the upregulation of mRNA for thyroid hormone receptors is unknown and can only be speculated on as to be caused by low tissue T3 in the myocardium of dogs with CHF. As there is no complete concordance between transcription of a certain gene and translation of the corresponding protein, it can only be hypothesized that increase in mRNA for the receptor eventually will result in an increase of the receptor itself. If so, there will be an increased responsiveness to thyroid hormone action in the myocardium. Whether or not it would be beneficial to employ this conceivable increased responsiveness to thyroid hormonal action, considering the effects of T3 on myocardial contractility, O_2 consumption, relaxation, hypertrophy, ANP production, and number of ß-adrenergic receptors,[57,58,264,265,274] or whether the altered expression of mRNA of myocardial TR subtypes should be viewed as a cardioprotective adaptation of the heart, is however not clear, and remains to be investigated.

CONCLUSIONS

The results of this thesis may be summarized as follows.

- There were no major differences concerning clinical, electrophysiologic, radiographic, echocardiographic, or histopathologic characteristics between the homogeneous group, i.e. Newfoundlands, and the heterogeneous group of dogs, which includes 38 breeds.

- Dilated cardiomyopathy (DCM) carries a poor prognosis in dogs, and only age and presence of dyspnea and ascites at time of diagnosis, can be used as prognostic markers. Young age at onset of clinical signs was the most significant risk factor identified.

- The histologic finding of attenuated wavy fibers has a very high sensitivity (99%) and specificity (100%) for canine idiopathic DCM.

- Sensitivity of standard clinical and echocardiographic criteria for the diagnosis of DCM is 93%, when final diagnosis is based on post mortem findings.

- Attenuated wavy fibers may develop before any clinical or echocardiographic signs of heart disease are evident, thus indicating an early stage of DCM, which may be denoted "occult DCM".

- PRA, PAC, urine aldosterone to creatinine ratio, and NT-proANP concentrations were significantly increased in dogs with symptomatic DCM, but not in dogs with asymptomatic DCM.

- Total thyroxine and T3 serum concentrations were not decreased in a majority of dogs with CHF caused by DCM. However, FT4 concentrations were significantly decreased in dogs with symptomatic DCM, compared to asymptomatic DCM dogs and normal control dogs. Messenger RNA for TR subtypes ß1 and ß2 were upregulated in dogs with heart failure attributable to DCM or CVD.

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