Detection of attenuated wavy fibers in the myocardium of Newfoundlands without clinical or echocardiographic evidence of heart disease

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Objectives—To determine whether attenuated wavy fibers may be found in the myocardium of Newfoundlands without clinical or echocardiographic evidence of heart disease.

Animals—15 Newfoundlands from a kennel with a known predisposition to dilated cardiomyopathy (DCM) and 32 dogs of other breeds that died suddenly or were euthanatized for reasons unrelated to heart disease and did not have gross postmortem evidence of heart disease.

Procedure—Echocardiography was performed on all Newfoundlands on a yearly basis. Necropsy specimens from all dogs were evaluated for attenuated wavy fibers (ie, myocardial cells < 6 μm in diameter with a wavy appearance).

Results—None of the Newfoundlands had clinical signs of heart disease, and results of echocardiographic examinations were within reference ranges. Seven Newfoundlands had histologic evidence of attenuated wavy fibers, whereas attenuated wavy fibers were not found in the remaining 8 Newfoundlands or in any of the 32 dogs of other breeds.

Conclusions and Clinical Relevance—Findings suggest that attenuated wavy fibers in dogs with a known predisposition for DCM may indicate an early stage of the disease. However, further studies on a larger number of dogs are needed to confirm this hypothesis. (*Am J Vet Res* 2000;61:238–241)

The diagnostic criteria for **dilated cardiomyopathy (DCM)** in dogs with clinical signs of **congestive heart failure (CHF)** are generally accepted to include low **fractional shortening (FS**; < 22% in Newfoundlands[1]) and dilatation of the cardiac chambers in the absence of other detectable cardiovascular disorders.[2-4] Dilated cardiomyopathy has been recognized in many medium-sized and large breed dogs,[3,4,a] with a high prevalence in Newfoundlands.[1,4,a] Subclinical DCM is commonly defined as echocardiographic evidence of left ventricular dilatation and hypokinesis in the absence of clinical signs of heart disease.[5,a,b]

The major histologic finding in dogs,[1,4,6,7] cats,[8] and humans[9,10] with DCM is the appearance of myocardial myocytes that are thinner than normal and have a wavy appearance, so-called attenuated wavy fibers. Detection of attenuated wavy fibers in the myocardium of dogs has been shown to have a high sensitivity (98%) and specificity (100%) for DCM.[11] The abnormal fibers are especially abundant subendocardially in the lateral wall of the left ventricle in dogs.[11]

The cause of DCM is presently unknown, although several theories concerning taurine[12,13] and carnitine[14] deficiency, antibodies against the β-receptor[15] or the adenine translocator,[16] myocarditis,[17] and mutations of the dystrophin gene[18] and the cardiac actin gene[19] have been proposed. In addition, the progression from cellular changes to clinical DCM is poorly understood. However, it has been suggested that development of attenuated wavy fibers may be secondary to chronic volume overload and stretching of myocytes in association with DCM.[9] It would, therefore, be of interest to determine the sequence of events involved in development of attenuated wavy fibers and echocardiographic evidence of chamber dilatation and myocardial hypokinesis. As a first step, the purpose of the study reported here was to determine whether attenuated wavy fibers may be found in the myocardium of Newfoundlands without clinical or echocardiographic evidence of heart disease.

Materials and Methods

Dogs—Medical records from the Albano Animal Hospital of Stockholm of 15 dogs (6 males and 9 females) participating in an ongoing survey of Newfoundlands from a breeding kennel with a known predisposition for DCM were reviewed. All 15 dogs had been euthanatized for reasons unrelated to heart disease. Age at the time of euthanasia ranged from 3 months to 9 years (median, 2 years; mean, 2.5 years). Body weight was recorded for 13 dogs and ranged from 14 to 61 kg (median, 46 kg; mean, 43 kg). All dogs had been examined clinically and by means of echocardiography on a yearly basis; however, 8 dogs were examined only once.

For comparison, results of complete postmortem examinations, including microscopic examinations, performed on 32 dogs (19 males, 13 females; mean age, 3.6 years) representing 23 breeds other than the Newfoundland breed that had died suddenly (n = 20) or were euthanatized (12) for reasons unrelated to cardiac disease were also included in the study.

Clinical evaluation—A physical examination, including cardiac auscultation, was performed on all Newfoundlands. M-mode and two-dimensional echocardiography were performed, using a 5 MHz transducer[c] placed on the right precordium, with dogs positioned in right lateral recumbency. Echocardiograms were recorded and analyzed according to recommendations of the American Society of Echocardiography[20] and the Echocardiographic Committee of the Speciality of Cardiology, American College of Veterinary Internal Medicine.[21] Previously published reference values[1,22] were used.

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Histologic evaluation—During postmortem examinations, hearts were examined grossly to exclude congenital defects, valvular disease, myocardial infarctions, and hemorrhage before collecting necropsy specimens for histologic examination. For the 15 Newfoundlands, necropsy specimens were collected from 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 lateral wall of the left ventricle, lateral wall of the right ventricle, and interventricular septum and from 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 10 specimens were examined. For the 32 non-Newfoundland dogs, necropsy specimens were collected from the lateral walls and papillary muscles of the left and right ventricles and from the left and right sides of the interventricular septum. Three slides from each of the 6 specimens were examined. Attenuated wavy fibers were defined as myocardial cells < 6 µm in diameter (reference range[23] for myofiber diameter, 10 to 20 µm) that had a wavy appearance. Dogs were considered to be positive for attenuated wavy fibers if at least half the thickness of the specimens from the proximal and distal portions of the left ventricular wall were composed of attenuated wavy fibers. All postmortem examinations, including histologic examinations, were performed by a single pathologist (LJ).

Statistical methods—Data are given as mean and SD. For continuous data that were normally distributed, groups were compared by use of Student's *t*-test for unpaired data. Variances were tested for equality between groups by use of the F-test (variance ratio test). When the F-test was significant (eg, age), nonparametric methods (eg, Mann-Whitney U test) were used. For categorical data, groups were compared by use of the χ^2 test. For all analyses, values of $P < 0.05$ were considered significant. All statistical calculations were performed by use of statistical software.[d]

Results

Reasons for euthanasia of the Newfoundlands included degenerative joint disease (n = 7), chronic dermatitis (2), hemopoietic disease (2), behavioral problems (2), chronic hepatic disease (1), and urolithiasis (1). Necropsy findings in the non-Newfoundland dogs included infectious or inflammatory disease (n = 20), neoplasia (7), trauma or bleeding (3), portosystemic shunting (1), and congenital renal dysplasia (1).

None of the Newfoundlands had a cardiac murmur during cardiac auscultation or any clinical signs of heart disease, such as dyspnea, tachycardia, or abdominal distension. Heart rate ranged from 60 to

Table 1—Signalment of and echocardiographic variables for Newfoundlands with (n = 7) and without (n = 8) evidence of attenuated wavy fibers in cardiac specimens obtained at necropsy

	Uncorrected values		Corrected values*	
Variable	Wavy fibers	Normal fibers	Wavy fibers	Normal fibers
Age (mo)	19.9 ± 14.6	41.5 ± 42.1	NA	NA
Sex (M/F)	3/4	3/5	NA	NA
Body weight (kg)	39.1 ± 19.9	47.9 ±14.2	NA	NA
Heart rate (beats/min)	133 ± 18†	112 ±12	NA	NA
LVEDD (mm)	40.1 ± 7.4†	48.3 ± 6.5	1.28 ± 0.60	1.17 ± 0.45
LVESD (mm)	27.6 ± 2.0†	35.5 ± 1.9	0.88 ± 0.44	0.85 ± 0.28
FS (%)	31.3 ± 6.3	26.9 ± 3.9	NA	NA
RV (mm)	11.6 ± 3.6	11.2 ± 3.5	0.38 ± 0.18	0.26 ± 0.06
LVWd (mm)	9.1 ± 2.3	8.4 ± 0.12	0.28 ± 0.13	0.21 ± 0.09
LVWs (mm)	12 ± 2.7	18 ± 0.17	0.37 ± 0.19	0.28 ± 0.10
LVW%	33.9 ± 11.6	34.7 ± 17.4	NA	NA
IVSd (mm)	8.1 ± 1.7	9.2 ± 2.8	0.26 ± 0.15	0.23 ± 0.08
IVSs (mm)	10.8 ± 1.6	11.8 ± 2.9	0.36 ± 0.20	0.30 ± 0.11
IVS%	35.9 ± 19.2	30.0 ± 9.1	NA	NA
La (mm)	21.6 ± 8.2	27.0 ± 2.0	0.81 ± 0.43	1.04 ± 0.65
Ao (mm)	20.2 ± 9.1	20.0 ± 2.6	0.74 ± 0.40	0.82 ± 0.65
La/Ao	1.1 ± 0.22	1.18 ± 0.13	NA	NA

*Values corrected for body weight. †Significantly ($P < 0.05$) different from value for dogs that did not have attenuated wavy fibers.

NA = Not applicable. LVEDD = Left ventricular end-diastolic diameter. LVESD = Left ventricular end-systolic diameter. FS = Fractional shortening. RV = Right ventricular diameter. LVWd = Left ventricular wall diastolic diameter. LVWs = Left ventricular wall systolic diameter. LVW% = Left ventricular wall percentage change. IVSd = Interventricular septum diastolic diameter. IVSs = Interventricular septum systolic diameter. IVS% = Interventricular septum percentage change. La = Left atrial diameter. Ao = Aortic root diameter. La/Ao = Ratio of left atrial to aortic root diameters.

Values are reported as mean ± SD.

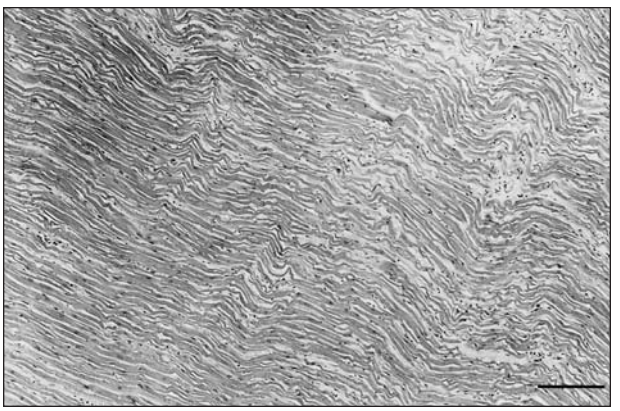

Figure 1—Photomicrograph of a section of myocardium containing attenuated wavy fibers from the left ventricle of a dog. H & E stain; bar = 200 µm.

Table 2—Results of histologic examination for attenuated wavy fibers in cardiac specimens obtained at necropsy from 7 Newfoundlands without clinical or echocardiographic evidence of heart disease

	Left ventricle				Right ventricle			Interventricular septum		
Dog No.	Proximal	Middle	Distal	Papillary muscle	Proximal	Middle	Distal	Proximal	Middle	Distal
1	+	+	+	+	–	–	+	+	–	+
2	+	–	+	+	+	+	+	+	–	–
3	+	+	–	+	–	+	–	+	+	–
4	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	–	–	–	+	+	+
6	+	+	+	+	+	+	+	+	–	+
7	+	+	–	+	+	–	+	–	–	+

+ = Positive results. – = Negative results.

149 beats/min (mean, 122.5 beats/min; **Table 1**). Left ventricular end-diastolic diameter ranged from 3.0 to 5.7 cm (mean, 4.4 cm; reference range, 3.5 to 6 cm), and left ventricular end-systolic diameter ranged from 2.1 to 4.2 cm (mean, 3.2 cm; reference range, 2.2 to 4.4 cm). Fractional shortening ranged from 22 to 40% (mean, 29%; reference range, 19 to 40%). The ratio between left atrial and aortic diameters ranged from 0.9 to 1.3 (mean, 1.2). Results of echocardiographic examinations were within reference ranges[1] for all Newfoundlands.

Seven of the 15 Newfoundlands were positive for attenuated wavy fibers (**Fig 1**, **Table 2**), but attenuated wavy fibers were not found in the remaining 8. Myocardial infiltrates of lymphosarcoma were found in 1 Newfoundland. None of the 32 non-Newfoundland dogs had attenuated wavy fibers or any other myocardial abnormalities.

When data were not corrected for body weight, heart rate, left ventricular end-diastolic diameter, and left ventricular end-systolic diameter were significantly different between Newfoundlands with attenuated wavy fibers and Newfoundlands without (Table 1). However, when echocardiographic variables were corrected for body weight, there were no longer significant differences between groups.

Discussion

In this study, attenuated wavy fibers were found in 7 of 15 Newfoundlands without clinical or echocardiographic evidence of heart disease; all of these dogs were from a breeding kennel with a known predisposition for DCM. Attenuated wavy fibers were not found in a control group of dogs that died suddenly or were euthanatized for reasons unrelated to heart disease. In combination with results of a previous study,[11] results of the present study suggest that development of attenuated wavy fibers is not a response to chamber dilatation and stretching of the myocytes. To the contrary, attenuated wavy fibers were detected in otherwise normal hearts. As attenuated wavy fibers have been shown to have a high specificity and sensitivity for DCM in dogs of various breeds,[4,11] results of the present study may suggest that development of attenuated wavy fibers represents an early stage of DCM, preceding development of clinical and echocardiographic signs of the disease. In addition, this characteristic histologic finding may be the result of a specific disease process in dogs with DCM, rather than the end result of many different pathologic processes, as has been suggested.[24] Unfortunately, myocardial biopsy specimens collected in vivo from the right ventricle may not be sufficient for identifying dogs with attenuated wavy fibers, as these abnormal myofibers were most abundant in the lateral wall of the left ventricle in dogs in the present study. Therefore, definitive diagnosis of DCM may necessitate postmortem examination of the heart or biopsy of the left ventricle.

The cause of attenuation, or atrophy, of myocytes in dogs with DCM is presently unknown. Atrophy is a common response of muscle fibers to processes that prevent normal contractile activity and to various pathologic stimuli.[25] Atrophy of cardiac myocytes has been shown to occur following prolonged mechanical support using left ventricular assist device systems[26] and following heterotopic isotransplantation (ie, transplantation of newborn mouse hearts into isogenic adult mice).[27] Myocardial atrophy has also been induced by hypothyroidism,[28] hypokinesia,[29] and malnutrition[30] in rats. Wavy myocardial fibers, especially when associated with focal edema, are a characteristic sign of acute myocardial ischemia in humans.[31] In humans with end-stage cardiomyopathy, development of attenuated wavy fibers seems to be reversible with unloading of the heart.[9]

Median and mean ages of the dogs in the present study were lower than mean and median ages of Newfoundlands with clinical disease,[1] and attenuated wavy fibers were even found in a 3-month-old puppy, suggesting an early onset of the disease process. Idiopathic DCM is considered to be hereditary in at least 20% of affected humans,[32] and the onset of clinical disease can be delayed for several decades.[33] It has recently been shown that a heritable form of DCM in humans is caused by mutations in the cardiac actin gene. Actin transmits the contractile force, generated by β-myosin heavy chains, cardiac troponin T, and other proteins, to adjacent sarcomeres and myocytes and to the extracellular matrix.[19] It has been proposed that idiopathic DCM in humans results from a defect in force transmission.[19] Such an impairment will prevent normal contractility and, thus, may cause myocyte atrophy.

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[b]O'Grady MR, Home R. Outcome of 103 asymptomatic Doberman Pinchers: incidence of dilated cardiomyopathy in a longitudinal study, in *Proceedings*. 13th Annu Vet Med Forum 1995;1014.

[c]Apogee, Interspec Inc, Advanced Technology Laboratory, Wash.

[d]JMP 3.2, SAS Institute Inc, Cary, NC.

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