DOBERMAN PINSCHER
HEALTH FOUNDATION

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The Doberman Pinscher Health Foundation is happy to inform you that we are funding the research titled "Spontaneous day-to-day variability of ventricular arrhythmias in overtly healthy Doberman Pinschers with normal echocardiographic parameters" in the amount of $7500.00.

Our group was founded by individuals touched by great tragedy and heartbreak. We were moved to start a group that would pool our resources and talents to help advance research to prevent others from suffering our same fate. We hope our support of your research will be another step to produce the knowledge and understanding to help our hearts heal and help someone else to prolong their dog's quality and quantity of life.

Sincerely,

Kelli Rosen DVM Chair DPHC Research Committee

Doberman Pinscher Health Foundation Grants Program Application

TITLE:

Spontaneous day-to-day variability of ventricular arrhythmias in overtly healthy Doberman Pinschers with normal echocardiographic parameters

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DURATION OF THE PROJECT AND TIMELINE:

It is anticipated that the number of dogs required for this study will be recruited within the first 6-12 months. We have collaborated with local Doberman breeder clubs and initiated patient recruitment. Data analysis and final report are expected to be completed within 12-18 months.

PROJECT DESCRIPTION:

1. Project Description in Lay language:

Dilated cardiomyopathy (DCM) is a devastating, highly prevalent (45-63% in USA and Canada), fatal heart disease that is common in Doberman Pinschers. Affected dogs develop progressive weakness and dilation of their heart chambers leading to poor pumping ability. The affected dogs go through at least two stages of the disease, the first being the preclinical stage in which dogs do not show any clinical signs. This is followed by the symptomatic stage when dogs present to the family veterinarian with signs of congestive heart failure. Within 6 months of diagnosis of DCM, most dogs die of congestive heart failure or experience sudden cardiac death even with the best available therapy. Recent studies have shown that treatment started in the early stages of DCM can significantly delay the onset of symptoms and extend survival duration. Consequently, early and accurate detection of Dobermans in the preclinical stage of DCM is of paramount importance so treatment can be initiated promptly. Various studies have shown that dogs in early stages of DCM develop abnormal heart rhythms even before the changes in heart muscle can be seen via echocardiogram. To date, 24-hour continuous ambulatory electrocardiography (24 hour Holter monitor) has been shown to have the best potential for detecting these dangerous heart rhythms (ventricular arrhythmias) in preclinical DCM. However, dogs, like people, are likely to experience wide variations in the day-to-day frequency of these arrhythmias. Therefore, depending on the day when the Holter recording was performed it could be possible to misdiagnose the severity of arrhythmia and miss the opportunity to start treatment early. At this time, it is unknown if any significant day to day variation exists in Dobermans during preclinical stage of DCM. The objective of this study is to evaluate multiple day Holter recordings for the presence of day-to-day variability in arrhythmia frequency in Dobermans during the preclinical stage of DCM. Using this preliminary information, we are also planning to conduct further longitudinal studies, evaluating the utility of multiple day Holter recordings in combination with other diagnostics to accurately predict disease progression in Dobermans with DCM.

2. Scientific Abstract:

Rationale: Dilated cardiomyopathy is a highly prevalent myocardial disease affecting Doberman Pinschers with two unique stages of the disease progression; the early, slowly progressive preclinical stage and the late onset, overt clinical stage characterized by onset of symptoms of congestive heart failure.[1-3] Diagnosis of dogs in preclinical stage of DCM is critical since treatment initiated prior to the development of clinical signs can significantly prolong survival duration in dogs.[4,5] Various studies have shown that dogs in preclinical DCM experience occult ventricular arrhythmias especially ventricular premature complexes (VPCs), the severity of which can predict future development of DCM.[6,7] Twenty-four hour Holter monitoring has been the gold standard for detecting occult VPCs during the preclinical stage of DCM.[8] However, in human patients and in Boxer dogs with arrhythmogenic cardiomyopathy, up to 80% day to day variation in VPC frequency is considered a normal variation.[9-10] If spontaneous variation also exists in Dobermans with DCM, it is likely to significantly affect the accuracy of a single 24 hour Holter.

Objective: The primary objective is to study the day-to-day variability of VPC frequency and complexity in overtly healthy Dobermans with normal echocardiographic results by studying the

number of positive and negative results for DCM prediction[6] observed between 24 hours and seven day Holter monitors.

Study design: Prospective, cross- sectional study

Animals and methods: Client owned, clinically normal Doberman dogs above 4 years of age with normal echocardiography will be prospectively recruited to the study and undergo seven day continuous Holter monitoring. The Holter recording will be analyzed for VPC frequency and complexity. The day-to-day variation in VPC frequency and complexity will be analyzed for each dog. The adequacy of 24 hour Holter monitoring to detect >50VPCs will be assessed using multiple day continuous Holter recording.

Expected outcomes: We expect that significant variation in day-to-day VPC frequency and complexity will be identified and multiple day Holter recordings will improve predictive value of Holter monitoring for DCM screening.

3. Hypothesis/Objective:

The objective of this study is to evaluate the day-to-day variability in VPC number and complexity during preclinical DCM in overtly health Dobermans with normal echocardiographic parameters using multiple day Holter monitoring.

4. Literature Review:

Dilated cardiomyopathy is one of the most common primary myocardial disease affecting Doberman Pinscher dogs.[1-2] It is an inherited, slowly progressive disease with prevalence as high as 58% in this breed.[3] The disease has a long preclinical stage during which most dogs remain asymptomatic. Once clinical signs develop, the disease progresses very quickly and affected dogs die of congestive heart failure or experience sudden cardiac death.[1-3] The preclinical stage is characterized by occurrence of ventricular arrhythmias, followed by progressive myocardial dysfunction and development of more severe forms of ventricular and atrial arrhythmias.[2] Sudden cardiac death presumably due to ventricular fibrillation has been reported in about 25-30% of the dogs with DCM irrespective of the echocardiographic findings.[7] About 37% of the dogs with DCM only have ventricular arrhythmias as an abnormality and often VPCs are the first detectable abnormality preceding echocardiographic evidence of DCM.[3] In addition, 29% of the dogs have both abnormal echocardiography and ventricular arrhythmias and 13% of dogs have only echocardiographic abnormalities.[3]

Various screening tools have been evaluated with the goal of diagnosing early stages of DCM and identifying animals at risk for developing DCM. A genetic test based on mutation of the pyruvate dehydrogenase 4 (PDK4) gene, encoding for a mitochondrial protein involved in energy metabolism has been developed in Dobermans with DCM.[12] Dogs that are positive homozygous or heterozygous for this PDK4 mutation are considered to be at increased risk for developing DCM.[12] However, due to variable penetrance of this gene, not all the dogs that have the mutation will eventually develop DCM. Additionally having a negative genetic test result does not preclude a dog from developing DCM in the future.[13] Biomarkers such as Cardiac Troponin (cTnI) and N-

terminal pro B-type natriuretic peptide (NT-proBNP) have been recently evaluated in Dobermans and offer some promise in improving diagnosis of preclinical DCM. However, NT-proBNP levels were most accurate in only detecting Dobermans that had echocardiographic changes during preclinical DCM stage and did not accurately identify dogs that had only ventricular arrhythmias.[14,15] Cardiac troponin levels were better in detecting dogs that had only ECG abnormalities but had poor sensitivity (79.5%) and specificity (84.4%) to detect any stage of DCM.[16] In addition, NT-proBNP and cTnI levels are not specific for DCM as they can be elevated in systemic disease, myocarditis and myocardial injury.[16] When biomarkers and genetic tests test results are combined with Holter monitoring or echocardiogram, the diagnostic accuracy is greatly improved.[16]

When echocardiography is normal during the preclinical stage of DCM, diagnosis depends solely on identifying occult ventricular arrhythmias on surface ECG. A recent study in Dobermans with DCM concluded that short term 5- minute ECGs were insensitive to detect VPCs compared to a 24-hour long term ambulatory ECG (Holter monitor).[11] Advanced high frequency short term ECG recording may be helpful to diagnose occult DCM but is not practically feasible in a clinical setting.[17] Currently, 24 hour Holter monitoring is the recommended gold standard test to detect occult VPCs during the preclinical stage of DCM.[8] Debate exists about the exact number of VPCs that are considered abnormal in Dobermans with preclinical DCM since the frequency of VPCs in normal Dobermans with out DCM is currently unknown. A longitudinal study using 24 hour Holter monitoring in healthy Dobermans with out echocardiographic evidence of DCM reported that the presence of more than 50 VPCs in a 24-hour period, or presence of 1 couplet or triplet is predictive for development of DCM.[6] Early screening for these occult ventricular arrhythmias is critical since therapy initiated during this time can be beneficial. A recent placebo controlled, multi center study in Dobermans with DCM has shown that therapy with Pimobendan during the preclinical stage can delay the onset of symptoms and increase median survival time.[5] Angiotensin converting enzyme inhibitors have also been shown to delay the progression of preclinical DCM to overt stage.[4] Therefore, early diagnosis of preclinical DCM is desirable so that therapy can be initiated promptly. In addition, early identification also helps in removing affected animals from breeding programs thereby decreasing the affected gene pool.

Even though the 24 hour Holter monitoring provides a better estimation of arrhythmia status at home and is superior to short term ECG's in detecting occult VPCs, it does have its own limitations. A study in people showed that longer term monitoring with 14-day novel continuous ECG patch monitors were more sensitive in detecting arrhythmias compared to a 24 hour Holter monitor.[18] This can be explained by the existence of wide spontaneous day to day variability in the frequency of VPCs in people independent of drug therapy.[19] Additionally, spontaneous variability is also reported in people with repetitive forms of ventricular arrhythmias such as couplets, triplets and non sustained ventricular tachycardia.[10] A seven day Holter study in boxer dogs diagnosed with arrhythmogenic right ventricular cardiomyopathy reported similar spontaneous variability to people.[9] The variability was especially higher in dogs with lower VPC frequency.[9] At this time it is unknown if day-to-day variability in VPC frequency exists in Dobermans with preclinical DCM. However, given the criteria of >50VPC/day for diagnosis of preclinical DCM even a small amount of day-to-day variation can significantly influence the diagnostic accuracy of 24 hour Holter recordings. Longer duration Holter monitoring over 24 hours is uncommon in veterinary patients and the majority of Holter studies in DCM dogs

evaluated only 24 hour Holter recordings. Therefore, there is paucity of information about the day to day variability in ventricular arrhythmia in Dobermans with any stage of DCM. Based on the preliminary observations from multiple day Holter recordings in preclinical Dobermans in our institution we predict that day to day spontaneous variability in VPC frequency exists in this group. Presence of significant day-to-day variability may affect the diagnostic accuracy of 24 hour Holter recordings depending on the day when the Holter recording is performed, which in turn can result in intolerable levels of false negative results.

5. Preliminary Studies supporting hypothesis:

We have observed the significance of day-to-day variability in a few clinical cases. A seven day Holter recording performed on a 4-year-old, male neutered, Doberman for routine DCM screening in our clinic revealed significant day-to-day variation in ventricular arrhythmia. This dog did not have any significant ventricular arrhythmias (range: 0-2 VPCs/24 hour, no couplets or triplets) during the first six days of the recording. However, on the seventh day of the recording there were 296 VPCs, 91 couplets and 225 triplets noted. If a single 24 hour Holter recording was performed in this dog, his Holter results would have been classified as being negative for preclinical DCM and therapy would have been delayed.

6. Anticipated Outcome and Significance:

We anticipate that significant spontaneous day-to-day variability will be detected in Doberman dogs with preclinical DCM and that multiple day Holter monitoring will improve diagnostic accuracy. Based on the results of this study, we are also planning to conduct further longitudinal studies, evaluating the utility of multiple day Holter recordings in combination with other diagnostics to accurately predict disease progression in Dobermans with DCM. This will ultimately result in earlier and more accurate diagnosis, prompt treatment initiation and overall improvement in quality of life and duration of the animal.

EXPERIMENTAL DESIGN: Prospective, cross-sectional study.

1. Animals and Treatment Protocols for Dogs:

Study dogs will be evaluated at College of Veterinary Medicine, Veterinary Medical Center, at Michigan State University, and receive routine cardiology examination including physical examination, transthoracic echocardiographic evaluation, and continuous Holter monitoring with seven-day recording capability. Transthoracic echocardiography will be performed using standard views as previously recommended for evaluation of DCM in Doberman Pinschers.[20] Approval from Michigan State University, Institutional Animal Care and Use Committee has already been obtained for this study.

Inclusion criteria:

- Client owned, Doberman dogs above 4 years of age with no reported clinical signs will be prospectively recruited to this study. Signed owners consent will be obtained prior to enrolment.
- Dogs with any NCSU DCM1 and NCSU DCM2 genetic test result status (negative, homozygous, and heterozygous) will be included in the study.
- Dogs with normal echocardiogram and previous 24-Holter results consistent with preclinical DCM will be considered for inclusion in the study provided that they are not receiving any medications or supplements that would affect the VPC frequency. If they are receiving therapy, enrollment will be considered after appropriate washout period as outlined in the methods section. The pros and cons of discontinuing supplements or drugs will be discussed with the owners prior to study enrollment.

Exclusion criteria:

- Dogs will be excluded if they have any systemic or chronic illness (hypothyroidism, auto immune disease, cancer, diabetes, Cushing's disease, liver disease, renal disease etc.) or receive any therapy (cardiovascular drugs: ACE inhibitors, spironolactone, pimobendan) antidepressants, anticonvulsants, antianxiety medications, antihistamines etc.) or receive any supplements (fish oil, taurine, L-carnitine etc.) that may affect the VPC frequency.
- Dogs will be excluded if they underwent any procedures requiring general anesthesia within the past 6 weeks.
- Dogs will be excluded if they have abnormal or equivocal echocardiogram results consisted with DCM. Echocardiographic values that will be considered normal: left ventricular internal end-diastolic dimension $\leq$ 47 mm, left ventricular internal end-systolic dimension $\leq$ 37 mm, and left ventricular fractional shortening $\geq$ 30%. Echocardiographic values that will be considered equivocal: left ventricular internal end-diastolic dimension between 48 and 50 mm, left ventricular internal end-systolic dimension between 38 and 40 mm, and left ventricular fractional shortening between 26 and 29%. Echocardiographic values that will be considered abnormal and consistent with DCM: left ventricular internal end-diastolic dimension > 50 mm, 9 left ventricular internal end-systolic dimension > 40 mm, and left ventricular fractional shortening $\leq$ 25%.
- Lactating, pregnant and animals in heat will also be excluded

2. Methods:

A minimum of a 6-week washout period is required if they are receiving any supplementation (i.e. fish oil, taurine, L-carnitine) or drugs (i.e. ACE inhibitors, spironolactone, pimobendan). Holter monitoring will be performed using three channel recorders, at a sampling frequency of 0.05Hz or 40Hz with orthogonal lead arrangement. The Holter monitors will be applied to the dogs at the time of their clinical appointment between the hours of 8am to 6pm using previously described techniques.[21] The dogs will be sent home for continuous ECG monitoring in their natural environment. The owners will be advised to keep a log of the dog's activities while wearing the device and note any clinical signs. The owners will be instructed to log every 2 hours documenting various activities including but not limited to sleeping, walking, running, feeding and any clinical signs noted such as collapse, weakness and anxiety. The data will be acquired at 1024 samples per second, stored on a 90MB flash card and transferred to a computer hard drive for automated analysis using a proprietary software.[a] The entire recording will be visually inspected by the cardiology resident (TG) under the supervision of a veterinary cardiologist (RAS) and will be corrected for any automated errors. Recordings that do not have at least 20-hour recording per 24-hour period will be excluded from the study.

3. Statistical significance/Statistician consultation:

Sample size determination:

No preliminary information is available about the daily frequency of VPCs and its day-to-day variability in Dobermans during the preclinical stage of DCM. Therefore, accurate sample size determination and power analysis could not be statistically performed. The individual variation in VPC frequency is expected to be large in this study group. We are planning to recruit 30 dogs initially for this pilot study. Based on the frequency of DCM in the breed, we expect to have at least 10 dogs meet the inclusion criteria.

Statistical methods:
Statistical significance will be set at alpha <0.05. Normality will be tested using Lilliefors test. Normally distributed variables will be represented with mean and standard deviation. Non normally distributed variables will be presented with median, range and IQR for the median when applicable. Categorical data will be represented as number of observations, percentages and ratio. For each dog, daily VPC frequency and frequency of other forms of VPC's will be calculated for the entire recording duration (maximum 7 days).

To compare single day and multiple day Holter recordings, positive DCM criteria will be defined as the presence of >50 VPCs, > 1 couplet, triplet or ventricular tachycardia (>4 VPCs in a row) in a 24-hour period. For each dog the number of positive DCM days out of total days will be tabulated. If a dog is negative on day 1 of the Holter recording but has at least one positive DCM criteria on any other day of the recording, it will be considered as failure of the 24-hour Holter recording. Proportion of success vs failure will be compared using a one-tailed dependent test for proportions.

REFERENCES OR LETTERS OF SUPPORT:

We have received past support from the Doberman Pinscher Club of America to fund the initial investigation.

BUDGET:

Budget Item	Per dog cost	Total cost for 30 dogs
1. Holter monitor supplies (includes dermal disposable gel electrodes, Holter vest, replacement leads, batteries and memory cards)	NA	$400.00
2. Echocardiography	$118	$3540.00
3. Multiple Day Holter monitoring (pays for technician time involved in placing Holter monitor, cost of obtaining Holter recorder that can be rotated among patients)	$100	$3000.00
Total :		$6940.00
4. Indirect costs		$555.00
Total amount requested		$7495.00

Holter recording evaluation and other technical work will be performed by the principal investigators (TG and RAS). No compensation for salaried personnel is requested at this time.

The proposed study does not evaluate any specific product or technology and the results are not expected to impact directly or indirectly any third party interests.

We are planning to seek additional funding for further studies based on the preliminary information gathered from this study.

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