ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)
Main Body
VERSION 4

See Instructions for Completion of the Animal Component of Research Protocol (ACORP Instructions), for help in completing specific items.

A. **ACORP Status.**

1. Full Name of Principal Investigator(s)► [redacted]

2. VA Station Name (City) and 3-Digit Station Number► **McGuire VAMC – Station 652**

3. Protocol Title► **Mechanistic Insight of Premature Ventricular Contractions-induced Cardiomyopathy**

4. Animal Species covered by this ACORP► **Canines**

5. Funding Source(s). Check each source that applies:

►( ) Department of Veterans Affairs.
►(X) US Public Health Service (e.g. NIH).
►( ) Private or Charitable Foundation -- Identify the Foundation:
►( ) University Intramural Funds – Identify the University and Funding Component:
►( ) Private Company – Identify the Company:
►( ) Other – Identify Other Source(s):

6. Related Documentation for IACUC reference.

   a. If this protocol applies to a project that has already been submitted to the R&D Committee for review, identify the project:

      (1) Title of project►

      (2) If approved by the R&D Committee, give the date of approval►

   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:

      (1) Identify the studies described in the previously approved ACORP that have already been completed
      ►

      (2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly
      ►

      (3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other Items below.
      ►

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).

(1) Title of other protocol ►
(2) IACUC approval number of other protocol ►
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):

►(X) Research
►( ) Teaching or Training
►( ) Testing
►( ) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of people and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.

►Premature heart beats are extra beats that present earlier than expected normal heart beats and can originate from the upper chamber. Animal and clinical studies have demonstrated that frequent upper chamber premature heart beats called "PVCs" (premature ventricular contractions) can diminish the squeezing function of the heart, also called "cardiac contractility". Moreover, the cardiac contractility may return to normal after elimination of PVCs. Unfortunately, we do not know the mechanism by which PVCs diminish cardiac contractility. The planned experiments will study the reduction in cardiac contractility caused by frequent PVCs. Frequent upper chamber premature heart beats will be replicated via an implantable pacemaker/defibrillator. An implantable pacemaker is a medical device that can produce heart beats on command. Similarly, an radio telemetry device will be implanted to continuously record the cardiac rhythm and nerve activity in response to premature heart beats and different medications.

The aim of this study is to understand the impact of PVC coupling intervals (expressed in milliseconds, between a normal sinus beat and the ensuing premature beat) in the development of Left Ventricle (LV) dysfunction and understand the mechanisms responsible for PVC and measurable deterioration of the ability of the heart muscle (myocardium) to contract, usually leading to heart failure known as cardio myopathy (CM). Our unique PVC canine model will allow us to study the pathophysiology of PVC-induced CM and understand the impact of coupling intervals to the development of this CM. Our frequent PVC and PVC-induced CM canine model will provide a systematic experimental approach to identify targets and eventual therapies to prevent PVC-induced CM and improve the management of patients with heart failure and PVCs.

C. **Experimental Design.**

1. **Lay Summary**. Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.

► This study will evaluate the reduction in cardiac contractility after frequent bigeminy premature heart beats (PVCs). Bigeminy is a descriptor for a heart arrhythmia in which there is a continuous alternation of a normal and premature heart beats. Most often this is due to heart beats occurring so frequently that there is one after each sinus normal beat. Frequent PVCs will be replicated in canines via an implantable pacemaker. Dogs have been chosen for this study due to the similarity to the human's heart electrical system and the canine model has been used extensively in cardiovascular research.

Under general anesthesia, all dogs will undergo open heart surgery to implant a pacemaker system in the right lower chamber as well as a radio telemetry device. In addition, catheters will be positioned on the heart surface to understand the changes in electrical signal with different extra beats. Finally, we will introduce catheters in the right and left heart chambers via small intravenous lines. These catheters will allow us to evaluate the changes in the right and left heart chamber pressures as well as pump function of the heart associated to frequent premature heart beat from the lower chambers. Ventricular programmed stimulation (VPS) will be performed through the implanted cardiac device to determine ventricular effective refractory period (VERP) and test susceptibility of malignant heart rhythm. The effective refractory period is the period of time where the cell is not responsive to stimuli therefore it is unable to contract. Left ventricular (LV) dyssynchrony is associated with frequent PVCs has never been studied. LV dyssynchrony is an abnormal contraction sequence of the heart that compromises its pump function. Baseline and changes in cardiac function contractility will be evaluated with blood samples during the treadmill challenge, biopsy during device implantation, biopsy at the end of the PVC 12 week period, biopsy collected at terminal surgery, and heart ultrasounds.

To observe how PVCs are affecting the animals' exercise capacity and possible arrhythmias, we will have the dogs complete 4 workouts on a canine treadmill. The true definition of heart failure states that symptoms are present at extreme or high levels of exertion.

2. **Complete description of the proposed use of animals.** Use the following outline to detail the proposed use of animals.

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.

► The chronic effects of post-extrasystolic potentiation and LV dyssynchrony associated with frequent PVCs have never been studied. The main purpose of this study is to use our premature pacing algorithm and PVC canine model to understand the triggers and mechanism responsible for PVC-induced CM.

To achieve our main goal, we plan to compare chronic states of frequent (ventricular bigeminy) short-coupled PVCs (coupling interval of 200-220ms), long-coupled PVCs (coupling interval of 320ms) and sham (without any ventricular ectopy). These PVC groups are chosen since long-coupled PVCs demonstrate more prominent LV dyssynchrony and lesser post-extrasystolic potentiation, while short-coupled PVCs display the opposite pattern, more prominent post-extrasystolic potentiation and lesser LV dyssynchrony.

Animal preparation & Pacemaker implantation

A total of 54 animals (see power analysis below) will undergo a left thoracotomy under general anesthesia to implant a bipolar epicardial ([redacted]) lead in the right ventricular (RV) apex connected to an experimental device ([redacted]). A two-biopotential channel and pressure transducer DSI transmitter ([redacted]) will be implanted subcutaneously to obtain ambulatory cardiac telemetry and blood pressure.

Group randomization

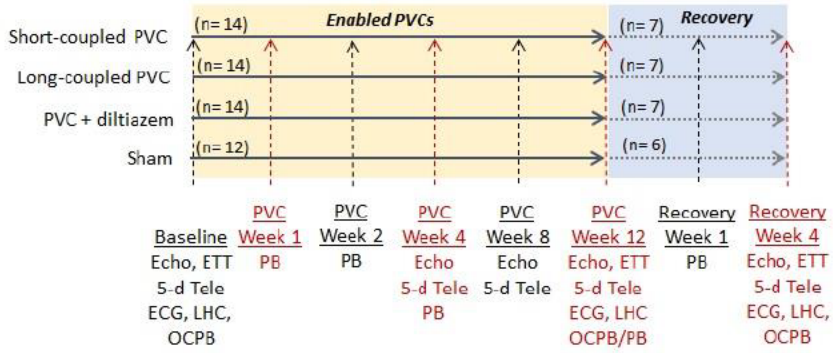

Figure 1. *Groups and procedures in a 12-wk PVC protocol and 4-wk recovery period (See text for details). ETT: Exercise treadmill test; 5-d Tele: 5-day ambulatory cardiac telemetry; LHC: left heart catheterization; ECG: electrocardiogram; VERP: Ventricular effective refractory period; OCPB: open chest punch biopsy; PB: percutaneous biopsy.*

After at least 2 week recovery period, all canines will be randomly assigned to 8 different groups: 1) short-coupled PVCs (200-220ms coupling interval, n=7), 2) short-coupled PVCs (200-220ms coupling interval with four week recovery, n=7) 3) long-coupled PVCs (320-340ms coupling interval, n=7), 4) long-coupled PVCs (320-340ms coupling interval with four week recovery,(n=7) 5) short-coupled PVCs + diltiazem 6mg/kg sustained release PO once a day for the duration of the study (n=7), 6) short-coupled PVCs + diltiazem 6 mg/kg sustained release PO once a day with a four week recovery (n=7) ,7) sham group (without PVCs, n=6) and 8) sham group with additional 4 weeks. (without PVCs, n=6). Regardless of group assignment, all animals will undergo baseline echocardiogram, ECG, 5-day cardiac telemetry, VERP and hemodynamic evaluation and open chest punch biopsy (OCPB) .After all baseline procedures are completed, device will be enabled to initiate 12-week exposure to randomized group (see section c below). Most animals will be euthanized after week 12, except for a few animals (6-7) who will be allowed a 4-week recovery phase (disabling PVCs) prior to euthanasia. If animal needs delayed on final surgery due to scheduling conflicts, animals will continue within the arrhythmia group assigned and no additional invasive procedures will be performed except for non-invasive echocardiogram. All animals regardless of randomization group, will undergo all procedures outlined in the figure.

Simulation of ventricular (PVCs) bigeminy.

[redacted] reproduce 50% PVC burden (bigeminy) based on two different prematurities: 1) [redacted] coupling interval (short-coupled PVC group), and 2) [redacted] (long-coupled PVCs group). As mentioned above, these prematurities were chosen due to the significant difference in LV dyssynchrony and post-extrasystolic potentiation (see underlined text above). Since the canine's heart rate ranges from 80-170 bpm, the proposed settings will achieve similar ectopic burden (bigeminal pattern) in both short- and long-coupled PVCs. Finally, sham group will have all pacing features disabled.

Myocardial biopsy.

All animals will undergo serial biopsies using a combination of open chest punch biopsy (LV free wall during pacemaker implantation) and percutaneous biopsy (PB) outlined in the figure. Percutaneous myocardial biopsies will be performed under fluoroscopy guidance using a 6 Fr Cordis Bipal 7 bioptome (5.2mm^3) to obtain 8 – 10 samples (the total of all 8-10 samples will be less than 1gm) from LV septum and free wall through a femoral approach. The biopsy samples will be snap frozen in liquid nitrogen and stored at -80∘C until analysis.
It is common to do weekly biopsies for up to 6 weeks on human heart transplant patients with complications related to the biopsy being very rare. Furthermore, these biopsies in humans are performed in the right ventricle which is thinner when compared to LV (which is what we are proposing in this canine PVC model).

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

► We estimate that the difference in the left ventricular ejection fraction (LVEF) of matched animals in the short- and long-coupled PVCs groups is normally distributed with SD of 6 % points. If the true difference in the mean LVEF between short- and long-coupled PVC groups is 7% points, 13 animals in each group will reject the null hypothesis that the means of LVEF in the short-coupled PVC group are equal to the long-coupled PVC group with probability (power) 0.85, and a Type I error probability of 0.05.
Our data has demonstrated a mild cardiomyopathy in early or short-coupled PVCs, where LVEF decreases from 60 to 40% after 3-month exposure. We hypothesize that late-coupled PVCs will have a lesser degree of CM, where the LV dysfunction may have 7% LVEF difference (decrease from 60 to 47% after 3 months) when compared to short-coupled PVCs. Thus, we need a sham group to assure that this mild CM is related to late-coupled PVCs and not to serial percutaneous biopsies. We estimate a 10% LVEF mean LVEF difference with a SD of 8 between late-coupled PVCs and no PVCs (Shams). Thus, we need 11 animals in sham group to differentiate the effects of late coupled PVCs and no PVCs (Sham).
We estimate that the LVEF difference of matched animals is normally distributed with standard deviation of 4.5 % points[18]. Assuming that animals have a true difference in the mean LVEF of 5% points between short-coupled PVC + Rx (diltiazem) vs. short-coupled PVC without Rx, we will need to study 13 animals in each group to reject the null hypothesis that the means of LVEF in the animals treated with diltiazem are equal to those without Rx (Power of 0.80, Type I error probability of 0.05).

Due to a 10% complication rate, an extra animal is added in each group (short-coupled PVCs with and without a recovery period n=14, long-coupled PVCs with and without a recovery period n=14, short-coupled PVCs + Rx without and without a recovery period n=14, sham n=12). Total animals needed: 54

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►

Initial Surgery

All canines will have an initial survival surgery where a pacemaker and radio telemetry device will be implanted via left thoracotomy. The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are given Buprenorphine 0.01-0.02 mg/kg IM, Penicillin (900,000 units) IM and Famotidine 0.5-1 mg/kg PO prior to being anesthetized. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. Average heart rate for the dogs under anesthesia is 85-100 bpm. If heart rate increases by more than 5bpm from the average to this point, isoflurane may be temporarily increase by .5% (to no more than 3% during surgery). Each dog will be weighed before surgery to determine the maximum dose of anesthetic that is allowed to prevent them from overdose

Left thoracotomy will be performed using sterile technique in the 4th intercostal space that will allow us to perform the following procedures:

1) Pacemaker implant: The heart will be suspended in a pericardial cradle to expose the RV apex. [redacted] **will be positioned and sutured with 2-0 silk in the RV apex or LV only if proper function is confirmed. A 2" incision will be made at the left dorsolateral area of the neck. Blunted dissection by planes will be performed until the musculature is reached. A 2-inch diameter pocket will be performed between the muscular fascia and subcutaneous tissue. The RV lead will be tunneled through the subcutaneous space to the subcutaneous pocket, where the lead(s) will be secured with a sawing sleeve to the muscular fascia with a 0-silk and connected to the device. The pacemaker will be positioned and sutured with a 0-silk to muscular fascia of the dorsolateral pocket. Appropriate device and lead function will be confirmed again prior to wound closure. The wounds will be closed in different planes with 1-0 Dacron and 0-silk.**

2) Radio telemetry device implant: A [redacted] **telemetry device will be implanted after pacemaker implant. The DSI device has two bipolar channels and one pressure transducer, which will be implanted to record (1) single lead ECG or left stellate ganglion nerve activity, (2) atrial electrogram or cardiac vagal nerve activity and (3) aortic pressure, respectively. The first two channels will be implanted through the thoracotomy after proper identification of specified structure. The pressure transducer will be introduced into the aorta through the left subclavian artery. The incisions will be closed in different planes with 1-0 Dacron and 0-silk.**

3) Hemodynamic evaluation (LHC): Animals will undergo an assessment of cardiac output, arterial blood pressure, pulmonary capillary wedge and left ventricular pressure while intubated and under general anesthesia. Using a percutaneous sledinger technique, two 6 French intravenous sheaths will be introduced in the right carotid artery and right external jugular vein. The former one will allow us to introduce a pigtail catheter to obtain LV pressures and LV pressure-volume loop recordings. The latter will allow introduction of a Swan-Ganz catheter. A 20GA IV catheter will be introduced into the right or left femoral arteries using a similar percutaneous technique. All Catheters will be connected to pressure transducers for continuous recordings. Hemodynamic assessment will include determination

of arterial blood pressure, mixed venous oxygen saturation, pulse pressure, cardiac output, cardiac index, LV end-diastolic pressure, LV pressure-volume loops, pulmonary pressure and pulmonary capillary wedge pressure. ECG will be monitored continuously during this procedure. Once perioperative hemodynamic data is obtained, venous and arterial sheaths will be removed and hemostasis will be obtained applying manual pressure. Attempt will be made to suture the puncture site in the carotid artery; however, ligation of the artery may be necessary if homeostasis is not achieved (as unilateral carotid ligation has performed safely in prior canine models, Udvary E, et.al, Br J Pharm 1995;114:656-661).

4) Electro-physiologic Study: Baseline Electro-physiologic study will be performed. Two multipolar adjustable Halo catheters (Irvine Biomedical Inc.) will be positioned in the epicardial surface of the base and mid-apical aspect of the RV and LV. Each ten-bipole catheter (0.5mm in diameter, 5 mm apart) will allow us to obtain local bipolar electrogram (EGM). The following electro-physiologic parameters will be obtained: 1) corrected QT interval (QTc); 2) ventricular effective refractory period (VERP); 3) monophasic action potential duration (MAPD) and interventricular MAPD dispersion (MAPD-D); 4) VERP/MADP90 ratio; 5) ventricular recovery time dispersion (VRT-D); and 6) ventricular late repolarization duration (VLRD). Baseline parameters will be acquired using a 32-channel Prucka GE Cardiolab electrophysiological system recording (General Electric, USA) during first survival left-thoracotomy for device implantation. All electro-physiologic parameters will be obtained during: 1) sinus rhythm and 2) PVCs from 3 different epicardial locations (RV apex, RVOT and LV free wall) and 4 different RV apical coupling intervals (200, 280, 400 and 600ms). Similarly, final parameters will be acquired at a final non-survival thoracotomy at the end of protocol in all groups (Figure 1).

5) Open Chest Biopsy: An open chest punch biopsy (LV free wall during pacemaker implantation) will be obtained from all animals. A 10-gauge core biopsy needle will be used to obtain 2-3 transmural biopsies, one from the anterior wall and second from LV free wall. Immediate after puncture, a single stitch with a 0-silk suture will be performed to obtain hemostasis. These biopsies will be obtained after all procedures above have been completed, prior to closing thoracotomy. This procedure carries a risk for malignant ventricular arrhythmias that could require cardiac resuscitation and could potentially result in sudden cardiac death.

There is a very small risk of spontaneous ventricular fibrillation during the left thoracotomy surgery. Should this happen there are sterile internal defibrillator paddlers connected to a defibrillator set at 50 Joules prepared for resuscitation efforts. Epinephrine will also be administered at a low dose (0.01 mg/kg) will be given every 3–5 min early in resuscitation efforts; a high dose (0.1 mg/kg) will be given after prolonged effort (15 minutes) with no response. Amiodarone (7mg/kg) will be used as an alternative. This is administrated in a single dose and repeated as needed every 5 minutes.

Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with Vetericyn gel to promote wound healing.The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/kg IM. The canine remains in the post-operative recovery cage until the following morning when they are moved to the standard chain link run with padding and blankets. They are given buprenorphine 0.01-0.02 mg/kg IM twice a day for three days for analgesia. Carprofen (2mg/kg PO) can be given for 1-3 days if pain is noted after the buprenorphine regimen is completed. Famotidine 0.5-1.0 mg/kg PO or IV can be given as needed for nausea or appetite stimulant (metoclopramide 0.2-0.5 mg/kg IV or bismuth subsalicylate 262 mg PO, can be given if needed). Some canines are too active post operatively which prevents incision healing. In this case, they are given Diazepam 0.2-2mg/kg IM or PO twice a day as needed. Cefpodoxime 5mg/kg PO is given once a day for

10 days to prevent wound infection. If there is ongoing concern for wound infection while receiving Cefpodoxime (Baytril 5-20mg/kg PO can be given once a day for 10 days). IV Fluids are available with or without 5% dextrose if an animal has not resumed normal eating habits within 2 days. Canine weights will be observed and recorded every other day while on antibiotics and then weekly until the animal completes the protocol.

Pacemaker interrogation

After pacemaker implantation, device will be interrogated every 1-2 weeks to assess for appropriate function, evaluating R wave amplitude (amplitude of ventricular signal), pacing thresholds, histograms and percentage of pacing. This will be performed via a St Jude Medical programmer. The programmer is and external device similar to laptop that has a "wand". The wand is positioned close to the device and allows pacemaker evaluation and programming. This process is not painful and will not represent any distress to the animal. We do not expect to require any type of restraint perform pacemaker interrogation. Pacemaker interrogation will last from 5 to 20 minutes depending on the findings.

Percutaneous Biopsy

All canines will undergo percutaneous biopsies (PB) post turning on PVC's on weeks 1, 2, and 4 to monitor the progression of LV dysfunction (Note that PVC's are turned on 2-4 weeks post first survival surgery). If there is a logistical conflict and / or transient medical contraindication to perform percutaneous biopsy, we will postpone biopsies as needed. No PBs will occur sooner than 7 days after previous PB.

Animals randomized to a recovery phase (see figure 1) will undergo the same number of biopsies, with an additional percutaneous biopsy at week 12 (when LV dysfunction is considered to be at its peak) . At this point the animals will have the PVCs turned off and allowed to recover for a month. A percutaneous biopsy will be performed at week 13 post PVC (week 1 of recovery).

The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes.

There is a very small risk of spontaneous ventricular fibrillation (VF) during the percutaneous biopsy. Amiodarone IV (5 mg/kg IV) will be administered prior to percutaneous biopsy by 20 minute infusion in an attempt to minimize this risk. Heparin IV 150-200 units/kg will be administered to prevent clot formation and possible embolic strokes. Arterial access will be obtained with either a Sledinger technique or direct arterial visualization performing a cut down in either carotid or femoral artery. A 6-9 Fr hemostasis sheath will be introduced over a guidewire guided by fluoroscopy. Percutaneous myocardial biopsies will be performed under fluoroscopy guidance using a 6 Fr Cordis Bipal 7 bioptome (5.2mm^3) thought the hemostasis sheath to obtain 8 – 10 samples (the sum of the 8-10 samples will be less than 1gm) from LV septum and free wall through a femoral or carotid approach. If cut down is performed, a direct closure of the vessel may be performed using a 5 to 7-0 silk under direct visualization. Alternatively, complete ligation of the artery may be performed if artery is considered to have a tear that is beyond repair. Wound will be closed by planes using Vycril 2-0 and 3-0 suture and anchor nylon interrupted mattress suture. Animal will be observed during a recovery phase of 2 hours. Diazepam (0.2-2.0 mg/kg PO or IM) can be administrated during the following 24 hrs in order to keep the animal calm. Though not common, if post biopsy pain is noted, Carprofen can be given 2mg/kg PO for 1-3 days.

The biopsy samples will be snap frozen in liquid nitrogen and stored at -80∘C until analysis. The nature of the multiple serial biopsies is to understand if the molecular changes (decrease in L-type Ca channel, Junctophilin-2 and BIN-1) demonstrated in our PVC-CM model are the cause or the result of the CM. This has been the "Achilles heel" of our publications, where reviewers question that our findings may not be the cause of the CM but rather the result of CM. In order to settle this question and support our findings and potential treatments to reverse this CM, we need to assess progressive molecular changes before CM starts. Thus, we plan to obtain PB at week 1, 2 and 4 (as PVC-CM is initiated based on our prior publication) and as this CM plateaus, which takes about 3 months. Furthermore, since PVC-CM is reversible, we need to demonstrate that these molecular changes reverse before the improvement in LV function. If our hypothesis is correct, this would clearly support the role of abnormal calcium handling as the cause of PVC-CM.

Hemodynamic evaluation: At the time of percutaneous biopsy, a Millar 5Fr pressure and impedance catheter will be introduced through 8Fr hemostasis sheath (used for PB) and advanced into the aorta and LV cavity. Hemodynamic recordings will be obtained from 1-5 minutes in each structure of interest.

Our proposal acknowledges the following Problems, Alternatives and Limitations:
1. Size limitation of a percutaneous biopsy (PB). The proposed studies are extensive for the potential small size biopsy obtained percutaneously as well as for the scope of this proposal. However, we feel ethically compelled to obtain as much data as possible, thereby minimizing number of future canines. Serial PB could potentially affect LV function. Thus, we have carefully chosen the number and time points. The sham group (without PVCs) will be key to assess the effects of serial PBs since they will not have any other distress. We plan to decrease the number of samples and interval between PB if shams demonstrate changes in LV function.
2. Molecular studies will be performed in the LV septum and free wall, while cell isolation will be performed only in the LV free wall since enzyme perfusion hampers the use of tissue block for other studies. Furthermore, simultaneous Langendorf preparation to obtain cell isolation from different LV sites it is quite challenging. Thus, regional differences will be only assessed by WB and qPCR.

Training
Most non-surgical procedures (echocardiograms, , electrocardiography, pacemaker interrogation and blood drawn) will be performed in a conscious state with minimal or no sedation. In order to achieve this, all animals will undergo training in order to lay or sit down still from 20-30 minutes at a time. This training will be performed by technicians. We estimate that this training will take from 2-4 weeks. Methods used for training will consist mostly on repetition with reward after completing different tasks, which will be gradually introduced and increased the duration of time until animals can lay or sit down for at least 30 minutes.

Echocardiogram
This non-invasive procedure is not painful and should not cause any distress to the animal. However, it requires that the animal stands still and possibly lays supine for at least 10 minutes in order to obtain accurate cardiac images. Therefore, we believe that 2 different approaches will be required to obtain echocardiogram: 1) animal training with or without restraint, and/or 2) general anesthesia. Our first approach will be animal training to stand and lay supine for 10 minutes to obtain echocardiogram. However, if the animal does not cooperate, we will first attempt to mildly sedate the animal with Acepromazine (0.05-0.1mg/kg) given PO approximately 1 hour prior to the procedure. If this is unsuccessful we will have to perform echocardiogram under general anesthesia with endotracheal intubation. We will use Brevital (6-10mg/kg) IV to effect (or Pentobarbital 30mg/kg, if Brevital is not available). Animals will be intubated, mechanically ventilated and anesthetized with

isoflurane 1-3%. After the echocardiogram, they will be allowed to recover from anesthesia in a post-operative recovery cage until able to walk to their run. No analgesics will be necessary due to the non-invasive nature of this procedure.

Baseline echocardiogram will be performed at least 2 weeks after pacemaker implantation and appropriate recovery of the animal. Subsequent echocardiograms will be performed on a monthly basis for the duration of the protocol. Echocardiogram will take from 10-20 minutes to obtain all required data. We estimate that each animal will undergo from 5 up to 12 echocardiograms depending on the assigned phase as per protocol.

Blood drawn
We will obtain blood sample in all groups at baseline as well on a monthly basis until the end of protocol. We will obtain no more than 10-15 cc, which represents less than 1% of body weight. We will plan to measure changes in atrial and brain natriuretic peptides as marker for heart failure. Blood will be drawn from the brachial or jugular veins.

Electrocardiogram (ECG).
Some animals will undergo baseline 6-lead ECG at least 2 weeks after pacemaker implantation and after baseline echocardiogram. Four patches will be attached to the skin to obtain lead V1, lead I, III, aVL, aVR and aVF. Proper preparation of patch position will be made by shaving the area and cleaning with alcohol pads any residual grease or debris that could interfere with proper attachment of patches. This procedure is estimated to last 5 minutes, it not considered painful and will not cause any distress to the animal. We do not expect to require any type of restraint to set up the ECG. After obtaining 6-lead ECG, all patches will be removed.

Ventricular programmed stimulation (VPS)
VPS will be performed through the implanted cardiac device to determine ventricular effective refractory period (VERP) and test susceptibility of ventricular arrhythmias. For VERP, a train of S1 at 400 and 300 ms cycle length is followed by S2 with 10 ms-decrement until S2 is unable to capture. Stimulus strength is twice the diastolic threshold. VERP is defined as the longest S1-S2 interval that does not elicit ventricular capture. For the evaluation of susceptibility to ventricular arrhythmias and drug effects, two 10-beat S1 trains at 400 and 300 ms cycle length with consecutive extra stimuli (S2 and S3) each with a gradual 10 ms-decrement until loss of capture is noted. VPES will be performed in a non-sedated state at baseline, after completion of PVC protocol (PVC group).
If sustained ventricular arrhythmias are induced, an external defibrillator will be in the prep room in order to resuscitate animal and restore normal rhythm. If defibrillation is required, animal will receive Carprofen 2 mg/kg daily for one to three days.
All animals will undergo VPS at baseline (2 weeks post-thoracotomy) and 1-7 days prior to final surgery.

Treadmill.
Canines will be exercised on a DogPACER (canine specific treadmill) to observe how PVCs are affecting the animals' exercise capacity, autonomic nervous/blood pressure recordings and possible arrhythmias. We have found that PVCs induced a mild cardiomyopathy, and yet, we cannot see physical findings of heart failure. For that reason, we need to be able to assess heart failure or decrease in exercise capacity in the canines. The true definition of heart failure states that symptoms are present at extreme or high levels of exertion. All animals thus far appear to be class I HF, but technically some of them may start at class I and later transition into class II HF.
To acclimatize the animals to the treadmill, they will initially be introduced by letting them explore the exercise room and equipment until they have become comfortable with those surroundings. Presence

of normal, relaxed behavior will signal that the dogs are ready for the next step, which is putting them on the treadmill while it's off. This will occur in small steps, putting them on for seconds and then extending the time. Each positive reaction will be rewarded with treats to encourage the dogs' learning process. When the dogs have become relaxed with the task of being on the still treadmill, they will next be put on the treadmill at its slowest speed, 0.5 mph. Two people will assist in this process; one person will hold the leash of the dog and stand in front of the treadmill offering rewards for positive behavior while the other will stand behind the animal making sure that she does not slide off of the machine, jump off of the sides and also to help the dog move their feet until she begins to understand and be comfortable with the movement herself. The process will take as long as needed to have the dogs become comfortable with the treadmill.
The treadmill workout will be done a total of 4 times in our study. The first 2 workouts will be performed 1-2 days apart at baseline about 2 weeks post-surgery after sutures have been removed. The final 2 treadmills will occur 1-2 days apart at the end of the study before final surgery. Each workout lasts 10 minutes, in which the dogs will complete 3 stages, each lasting 3 minutes. The first section will be at 1.1 mph followed by three minutes at 2.3mph and a final three minutes at 3.3 mph. Dogs can run 20-30 mph or more so this rate represents a fast walk to very slow jog.
We will determine fitness based on heart rate and serum lactate acid. Heart rate will be recorded before, at the peak of workout and at the finish. At that point, heart rate will be recorded every minute until it returns to baseline. The amount of time it takes for heart rate to return to baseline post-workout is the true measure of fitness. Heart rate can be displayed and monitored during the workout by a pacing analyzer that connects wirelessly to pacemaker implanted. In this way, we can also assess arrhythmias as the mild cardiomyopathy develops. Blood samples will be obtained through an IV catheter placed in the jugular or brachial vein. Blood will be drawn 4 times: once before, during each of 3 treadmill stages and recovery phase without exceeding 15mL (less than 1% of animal's body weigh). The blood will be drawn up through a syringe connected to a sterile intravenous catheter put in the jugular or brachial vein and skin taped comfortably around the dogs' necks or front leg to stay in place. After monitoring is done, the blood will be spun in a centrifuge and samples stored in -80 degrees Celsius until study at a later date.

<u>Final biopsy</u>
After 12 weeks of PVCs (when LV dysfunction is considered to be at its peak) the animals will undergo a final surgery involving a hemodynamic evaluation, electro-physiologic and open chest biopsy via a left thoracotomy. Animals randomized to the recovery phase will have final surgery after 4 weeks of recovery (post PVC week 16). The canine will be pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. Following left thoracotomy, all animals will undergo a final hemodynamic evaluation and electro-physiologic study (as described above in the initial surgery) followed by harvesting of the heart after euthanasia.

Samples will be obtained from the harvested heart after euthanasia at the end of a 12-week PVC period or after recovery phase if applicable. All serial and final biopsy samples will be snap frozen in liquid nitrogen and stored at -80∘C until analysis. Langendorf perfusion on an LV wedge will be performed.

Two additional and optional procedures may be required:

<u>Lead Revision</u>: If a pacemaker lead becomes dislodged, a lead revision surgery will be needed. We

will conduct his surgery either by thoracotomy. Procedures, anesthesia and analgesics would follow the same procedure as the initial surgery described above.

<u>Wound revision</u>: If the incision comes apart or the sutures do not hold the wound will have to be surgical closed. The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given if Brevital is unavailable) to effect and put on a mask with Isoflurane 2-4 % mixed with oxygen to maintain surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. They will be recovered in a post-operative recovery cage on a heated pad until able to walk to their run. Meloxicam will be given 2mg/kg IM after the surgery is complete. Carprofen 2mg/kg can be given for 1-3 days if needed. . Cefpodoxime 5mg/kg PO is given once a day for 10 days (alternatively if this isn't effective, Baytril 5-20mg/kg PO can be given once a day for 10 days)

D. **Species.** Justify the choice of species for this protocol.

► Canines have very similar physiology to humans. In addition there are significant differences in cardiac physiology between small animal species and humans. The experimental techniques, electronic pacemakers and leads available are large and require a larger species. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated large (approximately 2 inches long, ¼ inch thick and 1.5 inch wide) electronic defibrillator / pacemaker, which has been specifically developed for our study. The radio telemetry device is also large and will require internal implantation and observation for several months. Mostly biological pacemakers have been developed in smaller, less sentient species. In contrast to the electronic defibrillator / pacemaker, the biological pacemaker cannot modified its behavior easily, store and analyzed data. Moreover, an animal model with dogs has also been extensively studied in tachycardia-induced cardiomyopathy using an electronic pacemaker. Additionally, dogs have a His-Purkinje system located in endocardium, very similar to the human's heart, that pigs and other larger animals do not have.

<u>Personnel</u>

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.)

1. <u>PI</u>
   Name►[redacted]
   Animal research experience ► 6+ years with acute and chronic canines

   Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this ACORP
Cardiac perfusion and cell isolation	[redacted]
Pacemaker implants	

Canine handling and surgery	[redacted]
Electrocardiograms, Blood Draws, thoracotomy, pacemaker implantation	

2. Other research personnel (copy the lines below for each individual)

Name►[redacted]
Animal research experience ►[redacted]

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Thoracotomy	[redacted]
Inserting catheters, hemodynamic monitoring, implanting pacemakers	
Pacemaker implantation	
General canine surgery	

Name►[redacted]
Animal research experience ►[redacted]

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Cardiovascular surgery	[redacted]

Name►[redacted]
Animal research experience ► **She will serve as assistant animal care technician including surgical and all non-invasive procedures described in this project. She completed her Masters of Science in Laboratory Animal Sciences at [redacted].**

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Animal care	[redacted]
Assist in surgery and non-invasive procedures	

Name► [redacted]
Animal research experience ► [redacted]

Qualifications to perform specific procedures

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Animal care	[redacted]
Assist in surgery and non-invasive procedures	[redacted]

Name► [redacted]
Animal research experience ► [redacted]

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Canine cardiovascular surgery	[redacted]

Name► [redacted]
Animal research experience ► [redacted]

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Canine cardiovascular surgery	[redacted]

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual)

Name►

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course

Name of Individual	Working with the VA IACUC	ORD web-based species specific course (Identify the species)	Any other training required locally (Identify the training)
[redacted]	[redacted]		[redacted]

F. **Training to be provided.** List here each procedure in Item E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, enter "N/A"
► N/A

G. **Occupational Health and Safety.**

1. Complete one line in the table below for each of the personnel identified in Item E:

Name	Enrollment in OHSP		Declined optional services	Current on Interactions with OHSP? (yes/no)
	VA program	Equivalent Alternate Program – identify the program		
[redacted]	[redacted]		[redacted]	

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?

► ( ) Yes. Describe them ►

► (X) No.

Animals Requested

H. **Animals to be Used.** Complete the following table, listing the animals on separate lines according to any specific features that are required for the study (see ACORP Instructions, for guidance, including specific terminology recommended for the "Health Status" column):

Description (include the species and any other special features not shown elsewhere in this table)	Gender	Age/Size on Receipt	Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony)	Health Status
Canines, mongrel	**M/F**	**20-30 kg**	[redacted]	**Conditioned**

I. **Numbers of animals requested.** See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

USDA Category B

Procedures►						
Species / Experimental Group / Procedures(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category B TOTAL**

USDA Category C

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category C TOTAL**

USDA Category D

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category D TOTAL**

USDA Category E

Procedures►induced prolonged PVCs						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category E TOTAL**
Canine	**13**	**13**	**11**	**9**	**8**	**54**

TOTALS over all Categories

Species / Experimental Group /Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**GRAND TOTAL**
Canine	**13**	**13**	**11**	**10**	**8**	**54**

J. **Management of USDA Category D procedures**. Indicate which statement below applies, and provide the information requested.

► ( ) This protocol does NOT include any Category D procedures.

► (X) This protocol INCLUDES Category D procedures. List each Category D procedure and provide the information requested. (For surgical procedures described in Appendix 5, only identify the procedure(s) and enter "See Appendix 5 for details.)

Procedure	Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery)	Person(s) responsible for the monitoring	Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered)

Intial Surgery:Pacemaker implantation, Left Heart Catheterization (LHC), Hemodynamic evaluation, Open Chest punch biopsy	**electrocardiogram; See appendix 5 for details**	[redacted]	**Thoracotomy – 0.01-0.02 mg/kg IM Buprenorphine will be given before surgery for analgesia upon recovery. Brevital will be given IV prior to intubation and then surgical plane of anesthesia will be achieved with 1-3% Isoflurane inhaled until completion of surgery. Once the animal is sternal, Meloxicam 0.2mg/kg IM will be given followed by Buprenorphine 0.01-0.02 mg/kg IM the morning after surgery and continued BID for 3 days. Carprofen (2mg/kg) can be given for 1-3 days as needed.**

Percutaneous Myocardial Biopsy	**See appendix 5 for details**	[redacted]	**Animals will be anesthetized with Brevital (6-10 mg/kg) IV followed by inhaled 1-3% isoflurane to achieve a surgical plane of anesthesia which will be maintained throughout the surgery. Post biopsy pain is not common but Carprofen can be given 2mg/kg PO if needed. .**

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

► ( ) This protocol does NOT include any Category E procedures

► (X) This protocol INCLUDES Category E procedures. Identify each Category E procedure included in this ACORP and justify scientifically why the pain or distress cannot be relieved.
►

Ventricular Programmed Stimulation. Ventricular programmed stimulation (VPS) will be performed through the implanted cardiac device to determine ventricular effective refractory period (VERP) and test susceptibility of ventricular arrhythmias. The arrhythmias will lead to mild discomfort in the form of 'heart fluttering or racing' that may be distressful to the animal. There is no analgesic or anesthetic to alleviate this feeling and sedatives can interfere with the arrhythmias. This feeling will last 1-3 seconds. If sustained ventricular arrhythmias are induced an external defibrillator will need to be used to restore normal rhythm. If the external difibrillator is used, Carprofen will be administered 2mg/kg for 1-3 days afterward however this will not completely alleviate the pain from the defibrillation.

Veterinary Care and Husbandry

L. **Veterinary Support**.

1. Identify the laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

   Name►[redacted].
   Institutional affiliation►[redacted]
   email contact►[redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted►[redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ►[redacted]

M. **Husbandry.** As a reference for the animal husbandry staff, summarize here the husbandry requirements of the animals on this protocol. (Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals. Use Appendix 9 to document any aspects of the husbandry that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

1. Caging needs. Complete the table below to describe the housing that will have to be accommodated by the housing sites for this protocol:

a. Species	b. Type of housing*	c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)	e. Estimated maximum number of housing units needed at any one time
Canines	**Standard, see below**	**1**	**yes**	**17**

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:
► **Standard – raised floor, chain link, 3x6 feet minimum**

** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a justification if any animals will be housed singly (if species is not considered "social", then so note)
► **Dogs are housed singly in chain link runs, but can socialize as each room has at least 2 housing units that have no solid divider. Dogs are able to see and smell one another. Animals with enabled DSI transmitters (Turned "ON") will need to be housed singly in a cage for which DSI receivers are installed to receive signals from the transmitters. Mixing dogs will result in data cross talk. Pairs of dogs (even those with an enabled DSI transmitter) are allowed to exercise and play together in a designated "romper room. To avoid cross talk between different transmitters being picked up by a receiver, the dogs should only be let out to the activity room for "play time" one at a time, and always at a pre-specified time (so it is clear what period of time data will be lost or there is potential cross talk).**

***Use Appendix 9 to document "departures" from the standards in the *Guide*.

2. Enrichment. Complete the table below to indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required (See ACORP Instructions, for more information on enrichment requirements. Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards in the *Guide*.):

a. Species	b. Description of Enrichment*	c. Frequency
Canine	**Standard, see below**	**daily**

*If enrichment will be provided according to a local SOP, enter "standard (see SOP)" and enter the SOP into the table in Item Y. If the local standard enrichment is not described in a SOP, enter "standard, see below", and describe the standard species-specific enrichment here.
► **Daily milk bones, nylon chew bones rotated weekly, canvas toys**

3. Customized routine husbandry. Check all of the statements below that apply to the animals on this protocol, and provide instructions to the animal husbandry staff with regard to any customized routine husbandry needed.

► ( ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals, and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.
►

► ( ) Devices that extend chronically through the skin WILL be implanted into some or all animals on this protocol. Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.
►

► ( ) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.
►

► ( ) This ACORP does NOT include use of any animals that will require customized routine husbandry.

N. **Housing Sites**. Document in the tables below each location where animals on this protocol may be housed.

► ( ) Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU.

Building	Room number	Inside of VMU?	
		Yes	No
[redacted]	[redacted]	(X)	( )
		( )	( )
		( )	( )

► ( ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table.

Name of Non-VA Facility	Is this facility accredited by AAALAC?		Building	Room Number
	Yes -- enter status*	No**		
	( )	( )**		
	( )	( )**		
	( )	( )**		

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

Special Features

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".

► (X) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

► (X) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".

► ( ) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Q. **Locations of procedures.** Complete the table below, listing the location(s), inside or outside of the animal facility, for each of the procedures to be performed on animals on this protocol.

Procedure	Surgical?		Bldg/Room Number	Requires transport through non-research areas?	
	Yes	No		Yes – describe method of discreet transport	No
Pacemaker Implantation and Percutaneous myocardial Biopsy	**(X)**	**( )**	[redacted]	**( )**	**(X)**
Blood Draw	**( )**	**(X)**	[redacted]	**( )**	**(X)**
Echocardiogram	**( )**	**(X)**	[redacted]	**( )**	**(X)**
Electro-physiologic Study	**(X)**	**( )**	[redacted]	**( )**	**(X)**
Hemodynamic Evaluation	**(X)**		[redacted]	**( )**	**(X)**
Percutaneous myocardial Biopsy	**(X)**		[redacted]	**( )**	**(X)**
Treadmill		**X**	[redacted]		**X**

R. **Body Fluid, Tissue, and Device Collection.** List each body fluid, tissue, or device to be collected, and complete the table below to indicate the nature of the collection. Check the relevant Appendices in Item Y, below, and complete and attach them, as shown in the column headings.

Body Fluid, Tissue, or Device to be	Collected AFTER	Collected BEFORE Euthanasia

Collected	Euthanasia	Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production")	Collected as Part of a Surgical Procedure (Appendix 5, "Surgery")	Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection")
Blood	**( )**	**( )**	**( )**	**(X)**
Heart	**( )**	**( )**	**(X)**	**( )**
Pacemaker	**(X)**	**( )**	**( )**	**( )**
Percutaneous myocardial Biopsy	**()**		**(X)**	**X**
Open Chest Punch biopsy				**X**
Final biopsy	**X**			
Pacemaker and DSI device	**X**			

S. **Surgery.** Does this protocol include any surgical procedure(s)?

► (X) Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".

► ( ) NO animals on this protocol will undergo surgery.

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►Any animal that shows post-operative signs of pain or distress that cannot be controlled with analgesics will be euthanized. Signs of distress and pain will suspected in the presence of weight loss (> 12% body weight), lethargy, limping, vocalizing, excessive licking for 2-3 days and even aggression. Weights will be monitored every other day while animal is on antibiotics and then twice a week starting until the animal completes the protocol. If weight drop more than 5%, the vet will be notified and weights will be measured daily until completion of protocol or until weight returns to normal. This weight will be logged in the animals file
There is a very small risk of spontaneous ventricular fibrillation during the left thoracotomy surgery.

If after using the resurrection procedure with internal defibrillation paddles and epinephrine, the animal fails to respond, the animal will be euthanized using pentobarbital overdose and exsanguination.

There are rare risks associated with myocardial biopsies such as blood clots, bleeding, abnormal heart rhythm (ventricular tachycardia and fibrillation), infection, collapsed lung, injury to the artery and nerve, and rupture of the heart. These risks will be managed by performing this procedure in a sterile environment, providing antibiotics upon any sign of infection, keeping the animal anesthetized for at least 30 minutes post-surgery, and administrating diazepam post-surgery to keep the animal calm for 24 hours.

Similarly to thoracotomy, if there are any indications of the risks mentioned above the animal will be exsanguinated or terminated via pentobarbital overdose. If there is any evidence of these injuries post procedure, the animal will be immediately scheduled for a terminal surgery if possible.

U. **Termination or removal from the protocol**. Complete each of the following that applies:

► ( ) Some or all animals will NOT be euthanatized on this protocol. Describe the disposition of these animals. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these methods of disposition. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►

► (X) Some or all animals MAY be euthanatized as part of the planned studies. Complete the table below to describe the exact method(s) of euthanasia to be used. (Use Appendix 9 to document any departures from the standards in the *Guide* represented by these methods. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

Check each method that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable
( )	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death► Method for verifying death► Secondary physical method►		( )	( )	( )
(X)	Anesthetic overdose Agent►Pentobarbital Dose►100mg/kg Route of administration►IV	Canine	(X)	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )

(X)	Exsanguination under anesthesia Agent► **Isoflurane** Dose►**,1-3%** Route of administration► **inhale**	**canine**	(X)	( )	( )
( )	Other (Describe) ►		( )	( )	( )
( )	Other (Describe) ►		( )	( )	( )

1. For each of the methods above that is designated as "Conditionally Acceptable" by the AVMA, describe how the conditions for acceptability will be met:
   ►

2. For each of the methods above that is designated as "Unacceptable" by the AVMA, give the scientific reason(s) that justify this deviation from the AVMA Guidelines:
   ►

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.
   [redacted]

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
      ► **Carcass should be refrigerated and saved for autopsy. Staff should be contacted immediately.**

   b. Describe how the PI's staff should be contacted.
      ► ( ) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
      Name► [redacted]

► ( ) There is no need to contact the PI's staff immediately. Describe the routine notification procedures that will be followed. If the routine notification procedures are described in a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
►

V. **Special Procedures.** List each special procedure (including special husbandry and other special procedures) that is a part of this protocol, and specify where the details of the procedure are documented. See ACORP Instructions, for examples.

Name of Procedure	Identify Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP -- specify the Item letter(s)	Appendix 6
		Items:	()**
Radio Telemetry		Items:	(X)**
		Items:	( )**
		Items:	( )**

*If any special procedure is detailed in a SOP, identify the SOP and enter the information requested about the SOP in the table in Item Y.

**If any special procedure is detailed in Appendix 6, check "Appendix 6" in Item Y, below, and complete and attach Appendix 6.

(Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

W. **Consideration of Alternatives and Prevention of Unnecessary Duplication.** These are important to minimizing the harm/benefit to be derived from the work.

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ► Thoracotomy, percutaneous biopsy, blood draws, pacemaker implantation, lead revision

   Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
Medline	5/23/2017	2006-2017	Left ventricular dysfunction and PVCs. Autonomous nerve activity in PVCs	Premature ventricular contractions, cardiomyopathy, LV dysfunction, thoracotomy, percutaneous biopsy, Electrophysiologic study	(X)	(X)	(X)	(X)
ALTWEB	5/23/2017	2006-2017	Left ventricular dysfunction and PVCs, Autonomous nerve activity in PVCs.	Premature ventricular contractions, cardiomyopathy, LV dysfunction. thoracotomy, percutaneous biopsy, Electrophysiologic study	(X)	(X)	(X)	(X)
					( )	( )	( )	( )
					( )	( )	( )	( )

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.

► The study of changes in LV function and cardiac contractility due to frequent PVCs and PACs is unknown and therefore, there are no computer models to answer the unknown questions. We have recently described the first animal model of PVC-induced cardiomyopathy in canines. Otherwise, there are only studies in humans in whom frequent PVCs appear to affect cardiac contractility, however, there are multiple uncontrolled variables and thus clinical studies have multiple limitations.

The experimental techniques, electronic pacemakers and leads available are large and require a larger species. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated large [redacted] electronic defibrillator / pacemaker, which has been specifically developed for our study. This device will require internal implantation and observation for several months. Mostly biological pacemakers have been developed in smaller, less sentient species. In contrast to the electronic

defibrillator / pacemaker, the biological pacemaker cannot modified its behavior easily, store and analyzed data. Moreover, an animal model with dogs has also been extensively studied in tachycardia-induced cardiomyopathy using an electronic pacemaker, since dogs have a His-Purkinje system located in endocardium, very similar to the human's heart.

Pacemakers could be implanted using an intravascular technique as long as leads are placed in the right ventricle only. This technique would not allow implantation in the left ventricle due to risk of embolization/ stroke. However, DSI device has no alternative other than implantation via thoracotomy. Therefore, we perform implant of both (pacemaker and DSI) simultaneously during a single thoracotomy.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.
   ► The number of animals have been reduced to the minimum without compromising results as outlined in the power size estimation.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
   ► Our project is designed to minimize pain and distress, as well as use as few animals as possible. We will follow AWA recommendations to minimize distress and pain. Animals will have at least a week to acclimate to the new environment. Postoperatively, animals will receive analgesics and close monitoring to assess for any signs of pain or distress, such as weight loss, lethargy, limping, vocalizing, excessive licking and even aggression. In addition, animals will be trained technicians to undergo pacemaker interrogation and echocardiogram with minimal distress. The dogs will be handled daily to allow for maximum comfort and enjoyment of their environment.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
   ► Review of literature shows that this proposed study is novel and has never been performed previously.

X. **Other Regulatory Considerations**.

1. **Controlled drugs**.

   a. Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions, for explanations about the information requested.

Controlled substances	Storage		Personnel Authorized to Access	Location for Use		Procurement	
	Double-locked	Not Double-locked*		VA Property	Not on VA Property	VA Phar-macy	Non-VA
Brevital	(X)	( )*	[redacted]	(X)	( )	(X)	( )
Pentobarbital	(X)	( )*	[redacted]	(X)	( )	(X)	( )

Buprenorphine	(X)	( )*	[redacted]	(X)	( )	(X)	( )
Diazepam	(X)	( )*	[redacted]	(X)	( )	(X)	( )

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary.
►

b. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

► ( ) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy.

► (X) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.

► ( ) Other. Explain►

2. **Human patient care equipment or procedural areas**. Does this protocol involve use of any human patient care equipment or procedural areas?

► ( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".

► (X) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

3. **Explosive agents**. Does this protocol involve use of any explosive agent?

► ( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".

► (X) No explosive agent(s) will be used as part of this protocol.

Y. **Summary of Attachments.** To assist the reviewers, summarize here which of the following apply to this ACORP.

Appendices. Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

► ( ) Appendix 1, "Additional Local Information"
► ( ) Appendix 2, "Antibody Production"
► (X) Appendix 3, "Biosafety"

► (X) Appendix 4, "Ante-mortem Specimen Collection"
► (X) Appendix 5, "Surgery"
► (X) Appendix 6, "Special Husbandry and Procedures"
► ( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
► ( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
► ( ) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"

Standard Operating Procedures (SOPs). List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

Item	SOP		Approval Date
	Title	ID	
C.2.c			
M.1			
M.2			
U.4.a			
U.4.b			
V			

Z. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol. Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

   a. **Certification by Principal Investigator(s):**

   I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

   I understand that further IACUC approval must be secured before any of the following may be implemented:

   - Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
   - Changing any procedure in any way that has the potential to increase the pain/distress category

to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
- Performing any additional procedures not already described in this ACORP;
- Use of any of these animals on other protocols, or by other investigators.

I further certify that:

- No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC. When new or additional personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
- I will provide my after-hours contact information to the animal care staff for use in case of emergency.

Name(s) of Principal Investigator(s)	Signature	Date
[redacted]	[redacted]	[redacted]

b. **Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:

► ( ) No minority opinions were submitted by any IACUC participant for inclusion.

► ( ) Minority opinions submitted by IACUC participants are copied here
►

► ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages►)

Name of Attending Veterinarian (VMO or VMC)	Signature	Date

Name of IACUC Chair	Signature	Date

2. **Appendix 2. Antibody Production.** No signatures required.

3. **Appendix 3. Biosafety.**

   a. **Certification by PI(s) and IACUC Officials:**

   We certify that:

   - Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;

   - All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted]	[redacted]	[redacted]
Name of Institutional Veterinarian	Signature	Date
Name of IACUC Chair	Signature	Date

   b. **Certification by Biosafety Official.** I certify that:

   - Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is “toxic”, “infectious”, “biological”, or “contains recombinant nucleic acid”;

   - The use of each of the agents thus identified as “toxic”, “infectious”, or “biological”, or “contains recombinant nucleic acid” is further documented as required in Items 4, 5, 6, and/or 8, as

applicable, and in Item 10.a of Appendix 3;

- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee	Signature	Date

c. **Certification by Radiation Safety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "radioactive";
- The use of each radioactive agent is further documented as required in Items 7 and 10.a of Appendix 3;
- The use of each radioactive agent has been approved by the appropriate committee(s), as shown in Item 10.a of Appendix 3.

Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee	Signature	Date

4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.

5. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
- The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
- The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;

- The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
- Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:

    - Identification of each animal such that care for individual animals can be documented.
    - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
    - Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
    - Daily records covering at least the period defined as "post-operative" by local policy.
    - The signature or initials of the person making each entry.

.

Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted]	[redacted]	[redacted]

6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.

7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies.**

    a. **Certification by the Principal Investigator(s).** I certify that, to the best of my knowledge, the information provided in Appendix 7 of this ACORP is complete and accurate, and the use of patient care equipment or areas for these animal studies will be as described.

Name(s) of Principal Investigator(s)	Signature(s)	Date

    b. **Certification by the officials responsible for the use of any human patient care equipment in animal procedural areas.** Each of the following must sign to indicate that they have granted approval for the human patient care equipment to be moved to the VMU or other animal procedural area to be used on animals and then returned to the human patient care area, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of the Manager of the Human Patient Care Equipment	Signature	Date

c. **Certification by the officials responsible for the use of the equipment in human patient care areas for these animal studies.** Each of the following must sign to indicate that they have granted approval for animals to be transported into human patient care areas for study or treatment, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of Chief of Staff	Signature	Date
Name of Director or CEO of the Facility (Hospital or Clinic)	Signature	Date

8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals.**

a. **Certification by the Principal Investigator(s).**

I certify that, to the best of my knowledge, the information provided in Appendix 8 of this Animal Component of Research Protocol (ACORP) is complete and accurate, and the use of explosive agents in these animal studies will be as described.

I further certify that:

- Procedures involving explosive agent(s) will be performed within a properly operating, ventilated safety hood;
- All electrical equipment operating when explosive agent(s) are in use will be positioned and powered outside of the hood;
- Once the seal is broken on any containers of explosive agents, they will be kept in a safety hood throughout use, stored in an explosion-proof refrigerator or other approved storage area, and discarded properly once completely emptied;
- Proper procedures will be used for safe and appropriate disposal of items (including animal carcasses) that may contain residual traces of the explosive agent(s).

Name(s) of Principal Investigator(s)	Signature(s)	Date

b. **Certification by the officials responsible for overseeing the use of explosive agent(s) in this protocol.** Each of the following must sign to verify that they or the committee they represent have granted approval.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of Safety/Biosafety Officer for the Facility	Signature	Date
Name of ACOS for R&D	Signature	Date

Name of VISN Regional Safety Officer	Signature	Date

9. **Departures from "Must" and "Should" Standards in the *Guide*.** No signatures required.

ACORP APPENDIX 3
BIOSAFETY
VERSION 4

See ACORP App. 3 Instructions, for more detailed explanations of the information requested.

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:

Material (Identify the specific agent, device, strain, construct, isotope, etc.)	**Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors)	**Nature of Material**						
		Toxic Agent (Item 4)	Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4)	Biological Agent (Item 6)	Radioactive Agent (Item 7)	Contains Recombinant Nucleic Acid (Item 8)	Routine Pre- or Post-Procedural Drug	Euthanasia agent
Pentobarbital	Richmond VA pharmacy	( )					(X)	
Isoflurane	Richmond VA pharmacy	( )					(X)	
Brevital	Richmond VA pharmacy	( )					(X)	
Buprenorphine	Richmond VA pharmacy	( )					(X)	
Diazepam	Richmond VA pharmacy	( )					(X)	
Acepromazine	Butler Schein	( )					(X)	
Carprofen	Butler Schein	( )					(X)	
Baytril	Bayer	( )					(X)	
cefpodoxime	Butler Schein						X	
Penicillin	Butler Schein	( )					(X)	
Bismuth Subsalicylate	Local pharmacy						(X)	
Famotidine	Local pharmacy						(X)	
Meloxicam	Butler Schein						(X)	

Diltiazem	Richmond VA pharmacy						(X)	
Epinephrine	Richmond VA pharmacy						(X)	
Amiodarone	Richmond VA pharmacy						(X)	
Heparin	Richmond VA pharmacy						(X)	
Metoclopramide	VA Pharmacy						X	
DSI Device	Data Science International							
Pacemaker	[redacted]							
Vetericyn Gel spray	Butler Schein						X	

Summary of How Materials will be Administered. Complete the table below for each of the materials shown in the table in Item 1 above:

Material* (Identify the specific agent, device, strain, construct, isotope, etc.)	**Dose** (e.g., mg/kg, CFU, PFU, number of cells, mCi) and **Volume** (ml)	**Diluent* or Vehicle***	**Route** of admin	**Frequency or duration** of admin	**Reason** for Administration and **Expected Effects**	Location of **Further Details** in this ACORP (specify "Main Body" or "App #" and identify the Item)	Administration Under **Anesthesia, sedation, or tranquilization** (Y/N)
Pentobarbital	30mg/kg to effect or 100mg/kg for euthanasia	None	IV	Once for 1 day	Sedation during surgery and euthanasia	App5	Y
Brevital	6-10 mg/kg to effect	Normal saline	IV	Once per surgery	Sedation during surgery	App5	Y

Diazepam	0.2-2 mg/kg		PO or IM	As needed for sedation	Calm during post-op to keep sutures intact, and to keep animal calm post biopsy.	App5	N
Acepromazine	0.05-0.1mg/kg		Oral	Once per procedure	Calm for non-surgical procedures	App5	N
Carprofen	2 mg/kg		Oral	SID for 1-3 days or as needed	Anti-inflammatory and pain relief during post-op	App5	N
Baytril	5 – 20 mg/kg		Oral	SID for 10 days	Antibiotic during post-op, prevent infection	App5	N
Cefpodoxime	5 mg/kg	none	Oral	SID for 10 days	Antibiotic during post-op, prevent infection	App5	N
Isoflurane	1-4%		Inhalation	Continuous during surgery	Sedation during surgery	App5	Y
Penicillin	3 ml (300,000 units/ml)	None	IM	Once per thoracotomy	Antibiotic, prevent infection	App5	N
Buprenorphine	0.01-0.02 mg/kg	None	IM	BID for up to 3 days	Pain relief post-op	App5	N
Bismuth Subsalicylate	262 mg	None	Oral	Once a day as needed	Appetite recovery	App5	N
Famotidine	0.22-0.44 mg/pound	none	Oral	Once a day as needed	Appetite recovery	App5	N
Meloxicam	0.2 mg/kg	none	IM or SQ	Once after surgery or as an alternative for Carprofen	Pain Relief	App5	N
Diltiazem	6mg/kg	None	Oral	Once a day	treatment of hypertension	main	N

Epinephrine	Low dose (0.01 mg/kg) high dose (0.1 mg/kg)	Normal Saline	IV	every 3–5 min early in resuscitation efforts; after prolonged resuscitation efforts (15 mins)	Resuscitation efforts	App5	Y
Amiodarone	7mg/kg for resuscitation or 5 mg/kg IV bolus pre-biopsy	None	IV	over 10 min	Resuscitation efforts (alternative to epinephrine)	App5	Y
Heparin	150-200 units/kg	None	IV	Single bolus	Blood thinner to Prevent clot formation and embolic strokes.	App5	Y
Metoclopromide	0.2-0.5 mg/kg	none	IV	PRN	nausea	App5	N
DSI Device	None	None	SC implantation during first surgery	Once	Study device	App 5	Y
Pacemaker device	None	none	SC implantation during surgery	Once	Study device	App5	Y
Vetericyn Gel Spray	2-3 sprays	None	Topical	Once	To promote wound healing	Main body	N

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade. Check on-line for formulations that are FDA approved for administration to humans (http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm) or animals (http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847). Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, explain here why the use of a non-pharmaceutical grade formulation is necessary, and describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)
All items are USP

2. **Anesthesia, Sedation, or Tranquilization.** Complete 3.a. and 3.b. below:

   a. For each material with "Y" entered in the last column of the table in Item 2 above, describe the anesthesia, sedation, or tranquilization to be used, identifying the anesthetic, sedative, or chemical

tranquilizer, and detailing the dose, volume, and route of administration (Make sure that these agents are also included in Item 1 of this appendix, as materials to be administered):
► **Acepromazine (0.5-2.0 mg/kg PO) is given prior to the Brevital (or Pentobarbital) administration via IV catheter. The animal is sedated with Brevital or Pentobarbital prior to isoflurane administration. The DSI and Pacemaker are implanted under isoflurane anesthesia.**

b. For each material with "N" entered in the last column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.
►All agents given without anesthesia are administered via IM, IV or oral routes. Injections require no anesthesia as only momentary pain is experienced and none of the agents are irritating to the tissues. Agents given orally can be hidden in treats provided by the VMU or provided in a flavor tab that is eaten voluntarily by the canine.

3. **Toxic Agents.** Complete the table below for each of the materials listed as a "toxic agent" in the table in Item 1 above, checking the all of the properties that apply (see ACORP App. 3 Instructions, for details).

Name of Toxic Agent	a. Mutagen	b. Carcinogen	c. Teratogen	d. Select Agent?			e. Other – specify toxic properties
				Not a Select Agent	Select Agent Used in Sub-threshold Quantities	Select Agent that Requires Registration/Approval	
				()			()►anesthetic agent

*For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ►

Registered with CDC or USDA ►
Registration Number ►
Registration Date ►
Expiration Date of Registration ►

Name of official who granted approval on behalf of VACO►
Date of approval►

4. **Infectious Agents.** Complete the table below for each of the materials listed as an "infectious agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name and BSL Number of Infectious Agent	a. ABSL Number *	b. Drug Sensitivity Panel Available? (Describe)	c. Select Agent?		
			Not a Select Agent	Select Agent used in Sub-threshold quantities	Select Agent that Requires Registration/Approval
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**

*Complete the following for each agent for which the ABSL Number given is less than the BSL Number shown (copy the lines below for each agent):

Name of agent ►
Justification for applying ABSL measures that are less protective than those recommended ►

**For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ►

Registered with CDC or USDA ►
Registration Number ►
Registration Date ►
Expiration Date of Registration ►

Name of official who granted approval on behalf of VACO►
Date of approval►

5. **Biological Agents.** Complete the table below for each of the materials listed as a "biological agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Biological Agent	Screening for Infectious Agents

6. **Radioactive Agents.** Complete the table below for each of the agents listed as a "radioactive agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Radioactive Agent (specify the isotope)	Authorized Individual	Approving Committee or Official

7. **Agents Containing Recombinant Nucleic Acid.** For each of the materials checked in the table in Item 1, above, as "contains recombinant nucleic acid", indicate which of the conditions applies (see ACORP App. 3 Instructions, for details).

Name of Agent that Contains Recombinant Nucleic Acid	Subject to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*	Exempt
	( )	( )
	( )	( )

8. **Potential for Pain or Distress**. Complete the table below for each of the agents listed in Item 1, above, that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3 Instructions, for details).

Name of Agent	Nature of Potential Pain/Distress	Measures to Alleviate Pain/Distress

9. **Protection of Animal Facility Staff from Hazardous Materials.** Complete Items 10.a and 10.b, below, for each of the agents listed in the table in Item 1, above, as "toxic", "infectious", "biological", "radioactive", or "contains recombinant nucleic acid" (detailed in Items 4 – 8). This item specifically addresses members of the animal facility staff; protection of the research staff from each of these agents must be addressed in Item G of the main body of the ACORP. See ACORP App.3 Instructions, for details.

   a. Complete the table below.

Name of Hazardous Agent	Approving Committee or Official	Institution (VA or affiliate)	Names of Animal Facility Staff Members at Risk

b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents.
►

10. **Signatures.** Provide the applicable signatures on the signature pages (Item Z.3) of the main body of this ACORP.

ACORP Appendix 4
ANTEMORTEM SPECIMEN COLLECTION
VERSION 4

See ACORP App. 4 Instructions, for more detailed explanations of the information requested.

1. **Summary.** Complete the table below for each specimen to be collected from a live animal on this protocol (see ACORP App. 4 Instructions, for details).

Specimen Collected	Site and Method of Collection	Anesthesia (Yes/No)	Amount Collected Each Time	Volume Replacement (Yes/No/NA)	Total Number of Collections per Animal	Time Intervals Between Successive Collections
Blood	Brachial or Jugular vein/ Phlebotomy	No	10-15 ml (< 1%)	No	5	24 hours
Percutaneous Biopsy	C-arm guided Femoral / carotid access	Yes	8 – 10 samples (total of 8-10 samples will be less than 1gm)	NA	3-5 (dependent on experimental group)	7-14 days

2. **Use of Anesthetics, Tranquilizers, or Analgesics**.

   a. For each specimen described in Item 1, above, as being collected WITHOUT anesthesia, complete Items 2.a(1) and 2.a(2), below:

      (1) Explain why no measures will be taken to prevent pain (e.g., because of scientific requirements described here, or because the collection method involves no more than minor or momentary pain).
► No tranquilizers will be required for blood draws since the pain will be momentary. Administration of tranquilizers or aFacepronesthetics may cause similar discomfort or pain caused by phlebotomy.

      (2) Completely describe any method of physical restraint that may be used.
►

   b. For each specimen described in Item 1, above, as being collected WITH anesthesia, complete the following table:

Anesthetic, tranquilizer, or analgesic agent	Dose (mg/kg) and volume (ml)	Route of administration	Frequency of administration
Acepromazine	0.05-0.1mg/kg	Oral	Once
Brevital	6-10 mg/kg to effect	IV	Once

Isoflurane	2-4%	Inhalation	Continous during collection surgery
Diazepam	0.2-2 mg/kg	Oral or IM	Post biopsy if needed
Carprofen	2mg/kg	Oral	Post op if needed

3. **Volume Replacement for Fluid Collections.**

   a. For each fluid specimen described in Item 1, above, for which NO volume replacement will be provided, explain why not.
   ► Replacement is not necessary for the amount being drawn.

   b. For each fluid specimen described in Item 1, above, for which volume replacement WILL be provided, describe the replacement fluids that will be administered (including their composition, volume, and route of administration).
   ►

4. **Monitoring the animals.** Detail how the animals will be monitored after collection of specimens to ensure that they recover appropriately (see ACORP App. 4 Instructions, for details).
   ►[redacted] **monitor the dogs 5-10 minutes after blood draw to ensure they have recovered.**

   After heart tissue has been collected during the percutaneous biopsy, the animal will be monitored, sedated on the table after closing for 30 mins. After this, the animal will be moved to a recovery cage and monitored until sternal and then every 30 minutes for 2-3 hours after.

ACORP Appendix 5
SURGERY
VERSION 4

See ACORP App. 5 Instructions, for more detailed explanations of the information requested.

1. **Surgery Classification.** Complete the table below for each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). See ACORP App. 5 Instructions, for details.

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	**Initial Survival surgery/ pacemaker implantation and Open chest punch biopsy**	( )	( )	(X)	(X)*
2	**Percutaneous Biopsy**	( )	(X)	( )	(X)*
3	**Final Non-survival Protocol**	(X)	( )	( )	( )*
4	**Lead Revision**	( )	( )	(X)	(X)*
5	**Wound revision**		X		X

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:
►Percutanous Biopsy (PB). Thus far, cellular and molecular changes documented in our model have not been able to demonstrate causality of the CM. It has been questioned that this changes are due to the CM rather than the cause of it. PB will allow us to identify cellular and molecular changes that precede the development of LV dysfunction or so called "cardiomyopathy". Thus, it will allow to link cellular changes to the mechanism responsible for PVC-induced CM.
Thus, we plan to obtain PB at week 1, 2 and 4 (as PVC-CM is initiated based on our prior publication) and as this CM plateaus, which takes about 3 months. Those animals in the recovery group will have additional BP at week 12 and 13. Furthermore, since PVC-CM is reversible, we need to demonstrate that these molecular changes reverse before the improvement in LV function. If our hypothesis is correct, this would clearly support the role of abnormal calcium handling as the cause of PVC-CM.
Our proposal acknowledges the following Problems, Alternatives and Limitations:
Size limitation of a percutaneous biopsy (PB). The proposed studies are extensive for the potential small size biopsy obtained percutaneously as well as for the scope of this proposal. However, we feel ethically compelled to obtain as much data as possible, thereby minimizing number of future canines. Serial PB could potentially affect LV function. Thus, we have carefully chosen the number and time points. The sham group (without PVCs) will be key to assess the effects of serial PBs since they will not have any other distress. We plan to decrease the number of samples and interval between PB if shams demonstrate changes in LV function.
3. Molecular studies will be performed in the LV septum and free wall, while cell isolation will be performed only in the LV free wall since enzyme perfusion hampers the use of tissue block for other studies. Furthermore, simultaneous Langendorf preparation to obtain cell

isolation from different LV sites it is quite challenging. Thus, regional differences will be only assessed by WB and qPCR.
It is common to do weekly biopsies for up to 6 weeks on human heart transplant patients with complications related to the biopsy being very rare. Furthermore, these biopsies are performed in the right ventricle which is thinner when compared to LV (which is what we are proposing in this canine PVC model).

Lead Revision.This would only occur in the unique circumstance in which the pacemaker lead has unintentionally moved/dislodged. If this were to occur, animal will not provide scientific data for research, thus we believe that in some circumstances it would justify a second survival surgery to reposition the lead and have appropriate pacemaker function that would provide proper data for analysis.
Wound revision- it is not uncommon for surgical wound dehiscence to occur immediately after surgery. A minor and quick procedure would be needed to correct any defect.

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
►
Percutaneous Biopsy – Percutaneous Biopsies will not be performed until incisions from initial surgery have healed (14- to 28 days post). A minimum of 7 days between PBs. This is a minor procedure that should not have adverse effects with multiple time points.

Lead Revision- A minimum of 14 days to 8 weeks between the initial surgery and lead revision. This surgery will not be performed before the animal's incisions have fully healed.

Wound revision- This would occur 2-7 days after the initial surgery if needed.

2. **Description of Surgeries.** Describe each surgery listed in Item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

Surgery 1 ►
Initial Survival surgery: The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are given Buprenorphine 0.01-0.02 mg/kg IM, Penicillin (900,000 units) IM and Famotidine 0.5-1 mg/kg PO prior to being anesthetized. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given is Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. Average heart rate for the dogs under anesthesia is 85-100 bpm. If heart rate increases by more than 5bpm from the average to this point, isoflurane may be temporarily increase by .5% (no more than 3% during surgery). Each dog will be weighed before surgery to determine the maximum dose of anesthetic that is allowed to prevent them from overdose
Left thoracotomy will be performed using sterile technique in the 4th intercostal space that will allow us to perform the following procedures:
.

- **Pacemaker implant. The heart will be suspended in a pericardial cradle to expose the RA appendage and RV apex. Epicardial Medtronic (bipolar steroid eluting) lead (model No. 4968) will be positioned and sutured with 2-0 silk in the RV or LV only if proper function (as noted**

below) is confirmed.

Proper lead position will be defined as R wave greater than 4 mV, P wave greater than 2mV and a pacing threshold of less than 2 volts @ 0.5 msec. A 2" incision will be made at the left dorsolateral area of the neck. Blunted dissection by planes will be performed until the musculature is reached. A 2-inch diameter pocket will be performed between the muscular fascia and subcutaneous tissue. The RV lead will be tunneled through the subcutaneous space to the subcutaneous pocket, where the leads will be secured with a sawing sleeve to the muscular fascia with an 0-silk and connected to the device. The pacemaker will be positioned and sutured with an 0-silk to muscular fascia of the dorsolateral pocket. Appropriate device and lead function will be confirmed again prior to wound closure. The wounds will be closed in different planes with 1-0 Dacron and 0-silk..

- **Radio telemetry device implant. A [redacted] telemetry device will be implanted using the left thoracotomy performed during pacemaker implant (as above). The DSI device has two bipolar channels and one pressure transducer, which will be implanted to record (1) single lead ECG and 2) left stellate ganglion nerve activity or cardiac vagal nerve activity and (3) aortic pressure, respectively. The first two channels will be implanted through the thoracotomy after proper identification of specified structure. The pressure transducer will be introduced into the aorta through the left subclavian artery. The wounds will be closed in different planes with 1-0 Dacron and 0-silk.**

- **Hemodynamic evaluation (LHC). Animals will undergo an assessment of cardiac output, arterial blood pressure, pulmonary capillary wedge and left ventricular pressure while intubated and under general anesthesia with Isoflurane as mentioned above. Using a percutaneous sledinger technique, two 6 French intravenous sheaths will be introduced in the right carotid artery and right external jugular vein. The former one will allow us to introduce a pigtail catheter to obtain LV pressures and LV pressure-volume loop recordings. The latter will allow introduction of a Swan-Ganz catheter. A 20GA IV catheter will be introduced into the right or left femoral arteries using a similar percutaneous technique. All Catheters will be connected to pressure transducers for continuous recordings. Hemodynamic assessment will include determination of arterial blood pressure, mixed venous oxygen saturation, pulse pressure, cardiac output, cardiac index, LV end-diastolic pressure, LV pressure-volume loops, pulmonary pressure and pulmonary capillary wedge pressure. ECG will be monitored continuously during this procedure. Once perioperative hemodynamic data is obtained, venous and arterial sheaths will be removed and hemostasis will be obtained applying manual pressure. Attempt will be made to suture the puncture site in the carotid artery; however, ligation of the artery may be necessary if homeostasis is not achieved (as unilateral carotid ligation has performed safely in prior canine models, Udvary E, et.al, Br J Pharm 1995;114:656-661).**

- **Electro-physiologic Study. Baseline Electro-physiologic study will be performed.**
  Two multipolar adjustable Halo catheters (Irvine Biomedical Inc.) will be positioned in the epicardial surface of the base and mid-apical aspect of the RV and LV (Figure 6). Each ten-bipole catheter (0.5mm in diameter, 5 mm apart) will allow us to obtain local bipolar electrogram (EGM).
  The following electro-physiologic parameters will be obtained: 1) corrected QT interval (QTc); 2) ventricular effective refractory period (VERP); 3) monophasic action potential duration (MAPD) and interventricular MAPD dispersion (MAPD-D); 4) VERP/MADP90 ratio; 5) ventricular recovery time dispersion (VRT-D); and 6) ventricular late repolarization duration

(VLRD). Baseline parameters will be acquired using a 32-channel Prucka GE Cardiolab electrophysiological system recording (General Electric, USA) during first survival left-thoracotomy for device implantation.
All electro-physiologic parameters will be obtained during: 1) sinus rhythm (disabled PVC/PAC algorithm), 2) PVCs from 3 different epicardial locations (RV apex, RVOT and LV free wall) and 4 different RV apical coupling intervals (200, 280, 400 and 600ms), and 3) PACs (enabled algorithm) to achieve 280ms R-R interval. Similarly, final parameters will be acquired at a final non-survival thoracotomy at the end of protocol in all groups (Figure 1).

- **Open Chest punch biopsy. Following the thoracotomy surgery, open chest punch biopsy (LV free wall during pacemaker implantation) will be obtained from all animals. A 10-gauge core biopsy needle will be used to obtain 2-3 transmural biopsies, one from the anterior wall and second from LV free wall. Immediate after puncture, a single stitch with a 0-silk suture will be performed to obtain hemostasis. These biopsies will be obtained after all procedures above have been completed, prior to closing thoracotomy. This procedure carries a risk for malignant ventricular arrhythmias that could require cardiac resuscitation and could potentially result in sudden cardiac death.**

There is a very small risk of spontaneous ventricular fibrillation during the left thoracotomy surgery. Should this happen there are sterile internal defibrillator paddlers connected to a defibrillator set at 50 Joules prepared for resuscitation efforts. Epinephrine will also be administrated.at a low dose (0.01 mg/kg) every 3–5 min early in resuscitation efforts; high dose (0.1 mg/kg) will be given after prolonged effort (around 15 minutes) with no response. As an alternative to epinephrine, amiodarone (7 mg/kg IV over 30 60 minutes) will be used.
Pneumothorax will be prevented by ensuring that the chest is properly and tightly closed and is air tight at the end of the procedure.
Animals are allowed to recover on the ventilator with 100% oxygen until swallowing reflex is noted. The incisions are sprayed with a Vetericyn Gel to promote wound healing. The endotracheal tube is removed and the canine is moved to a recovery cage with blankets and a warming pad. Once sternal, they receive meloxicam 0.2mg/kg IM.
The canine remains in the post-operative recovery cage until the following morning when they are moved to the standard chain link run with padding and blankets. They are given buprenorphine 0.01-0.02 mg/kg IM twice a day for three days for analgesia. Carprofen (2mg/kg PO) can be given for 1-3 days if pain is noted after the buprenorphine regimen is completed. Famotidine 0.5-1.0 mg/kg PO or IV can be given as needed for nausea or appetite stimulant (metoclopramide 0.2-0.5 mg/kg IV or bismuth subsalicylate 262 mg PO, can be given if needed). Some canines are too active post operatively which prevents incision healing. In this case, they are given Diazepam 0.2-2mg/kg IM or PO twice a day as needed. Cefpodoxime 5mg/kg PO is given once a day for 10 days (alternatively if this isn't effective, Baytril 5-20mg/kg PO can be given once a day for 10 days)
The dogs will be allowed to recover for 2 weeks prior baseline echocardiogram and initiation of High PVC burden protocol.

Canine weights will be observed and recorded every other day while on antibiotics and then twice a week for the duration of the study.

Surgery 2 ► Percutaneous Biopsy.

Each animal will undergo at least 3 percutaneous biopsies (PVC weeks 1, 2, and 4). Animals who are part of the recovery phase (6-7 animals per experimental group) will undergo 2 more (PVC week 12 and Recovery week 1). Percutaneous myocardial biopsies will be performed under fluoroscopy guidance using a 6 Fr Cordis Bipal 7 bioptome (5.2mm^3) to obtain 8 – 10 samples (the total of the 8-10 samples will be less than 1gm) from LV septum and free wall through a femoral approach. The biopsy samples will be snap frozen in liquid nitrogen and stored at -80∘C until analysis.

The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given is Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes.

There is a very small risk of spontaneous ventricular fibrillation (VF) during the percutaneous biopsy. Amiodarone IV (5 mg/kg IV) will be administered prior to percutaneous biopsy by 20 minute infusion in an attempt to minimize this risk. Heparin IV 150-200 units/kg will be administered to prevent clot formation and possible embolic strokes. Arterial access will be obtained with either a Sledinger technique or direct arterial visualization performing a cut down in either carotid or femoral artery. A 6-9 Fr hemostasis sheath will be introduced over a guidewire guided by fluoroscopy. Percutaneous myocardial biopsies will be performed under fluoroscopy guidance using a 6 Fr Cordis Bipal 7 bioptome (5.2mm^3) thought the hemostasis sheath to obtain 8 – 10 samples (the sum of the 8-10 samples will be less than 1gm) from LV septum and free wall through a femoral or carotid approach. If cut down is performed, a direct closure of the vessel may be performed using a 5 to 7-0 silk under direct visualization. Alternatively, complete ligation of the artery may be performed if artery is considered to have a tear that is beyond repair. Wound will be closed by planes using Vycril 2-0 and 3-0 suture and anchor nylon interrupted mattress suture. Animal will be observed during a recovery phase of 2 hours. Diazepam (0.2-2.0 mg/kg PO or IM) can be administrated during the following 24 hrs in order to keep the animal calm. Canine weights will be observed and recorded every other day while on antibiotics and then twice a week until the animal completes the protocol. Though not common, if post biopsy pain is noted, Carprofen can be given 2mg/kg PO for 1-3 days.

Hemodynamic evaluation. A Millar 5Fr pressure and impedance catheter will be introduced through 8Fr hemostasis sheath (used for PB) and advanced into the aorta and LV cavity. Hemodynamic recordings will be obtained from 1-5 minutes in each structure of interest.

During this procedure, there is a small risk of malignant ventricular arrhythmias that could lead to unexpected death. To minimize the risk, we will maintain vital signs stable with proper support including oxygenation and provide 5mg/kg Amiodarone IV bolus via slow infusion prior to the procedure. In the event that this complication occurs, we will be prepared with an external defibrillator and proper advanced life cardiac support (ACLS) medications such as epinephrine and amiodarone.

After procedure is completed, hemostasis sheath will be removed over a wire and hemostasis obtained with manual pressure for at least 20-30 minutes. In the event of a hematoma, pressure will be maintained for longer. Animal will be monitored during a recovery phase of 4 hours.
They will be moved to a post-operative recovery cage with a warming pad until they are able to walk to their run. Carprofen can be given for post-operative pain but this is not common with this procedure.

Surgery 3 ► Final non-survival protocol

After completing the PVC burden protocol, all animals will undergo a final hemodynamic evaluation and electro-physiologic study (as described above in initial surgery) via a left thoracotomy during a non-survival surgery. The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given is Brevital is unavailable) to effect to allow for intubation with a cuffed endotracheal tube. The endotracheal tube is then connected to a vaporizer and respirator for isoflurane induction and mechanical ventilation. Isoflurane 1-3 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes.

A left thoracotomy is performed and the following procedures are conducted during this non survival surgery:

- **Hemodynamic evaluation. During the final surgery, animals will undergo an assessment of cardiac output, arterial blood pressure, pulmonary capillary wedge and left ventricular pressure while intubated and under general anesthesia with Isoflurane as mentioned above. Using a percutaneous sledinger technique, two 6 French intravenous sheaths will be introduced in the right carotid artery and right external jugular vein. The former one will allow us to introduce a pigtail catheter to obtain LV pressures and LV pressure-volume loop recordings. The latter will allow introduction of a Swan-Ganz catheter. A 20GA IV catheter will be introduced into the right or left femoral arteries using a similar percutaneous technique. All Catheters will be connected to pressure transducers for continuous recordings. Once atrial and ventricular leads are implanted, frequent PVCs and PACs will be enabled via pacemaker to assess hemodynamic changes. Hemodynamic assessment will include determination of arterial blood pressure, mixed venous oxygen saturation, pulse pressure, cardiac output, cardiac index, LV end-diastolic pressure, LV pressure-volume loops, pulmonary pressure and pulmonary capillary wedge pressure. ECG will be monitored continuously during this procedure. Once perioperative hemodynamic data is obtained, right IJ venous sheaths and the femoral arterial line will be removed and hemostasis will be obtained applying manual pressure. Attempt will be made to suture the puncture site in the carotid artery; however, ligation of the carotid artery may be necessary if homeostasis is not achieved (as unilateral carotid ligation has performed safely in prior canine models, Udvary E, et.al, Br J Pharm 1995;114:656-661). The pigtail and Swan-Ganz catheters will be reused after appropriate chemical re-sterilization with glutaraldehyde.**

- **Electro-physiologic Study. A final electro-physiologic study will be performed via left thoracotomy during final non-survival surgery.**
  Two multipolar adjustable Halo catheters (Irvine Biomedical Inc.) will be positioned in the epicardial surface of the base and mid-apical aspect of the RV and LV (Figure 6). Each ten-bipole catheter (0.5mm in diameter, 5 mm apart) will allow us to obtain local bipolar electrogram (EGM).
  The following electro-physiologic parameters will be obtained: 1) corrected QT interval (QTc); 2) ventricular effective refractory period (VERP); 3) monophasic action potential duration (MAPD) and interventricular MAPD dispersion (MAPD-D); 4) VERP/MADP90 ratio; 5) ventricular recovery time dispersion (VRT-D); and 6) ventricular late repolarization duration (VLRD). Final parameters will be acquired using a 32-channel Prucka GE Cardiolab

electrophysiological system recording (General Electric, USA) during first survival left-thoracotomy for device implantation.
All electro-physiologic parameters will be obtained during: *1) sinus rhythm (disabled PVC/PAC algorithm), 2) PVCs from 3 different epicardial locations (RV apex, RVOT and LV free wall) and 4 different RV apical coupling intervals (200, 280, 400 and 600ms), and 3) PACs (enabled algorithm) to achieve 280ms R-R interval.*

- **Final biopsy. Following completion of the diagnostic studies the animal undergoes euthanasia through exsanguination under anesthesia with harvesting of the heart. The samples will be obtained from the harvested heart after euthanasia at the end of a 12-week PVC period or after recovery phase if applicable. All serial and final biopsy samples will be snap frozen in liquid nitrogen and stored at -80∘C until analysis. Langendorf perfusion on an LV wedge will be performed.**

Surgery 4 ► **Lead revision. This is a surgical procedure to be performed only in those animals that have already undergone pacemaker implantation that happened to have a lead dislodgement of either the RA or the RV lead. This lead revision will be done via thoracotomy. Procedures, surgical technique and recovery would be the same as section "a".**

Surgery 5► Wound revision. Canines will on occasion be subject to surgical wound dehiscence. These animals will be returned to the OR for repair of the dehiscent wound, and drainage of any infectious or noninfectious fluid collections. . The canine is pre-anesthetized with Acepromazine 0.05-0.1mg/kg approximately 1 hour before surgery. They are anesthetized with Brevital 6-10 mg/kg IV (Pentobarbital 30mg/kg IV can be given is Brevital is unavailable) to effect and placed on a mask for isoflurane induction. Isoflurane 2-4 % mixed with oxygen is used for surgical plane of anesthesia throughout the surgery unless otherwise described. Heart rate, blood pressure and temperature will be recorded every 15 minutes. The procedure will be less than 30 minutes. The animal will recover in the post-operative recovery cage with a warming pad until they can walk to their run. They will be given Meloxicam 0.2 mg/kg IM after surgery and Cefpodoxime 5mg/kg PO for 10 days.

3. **Personnel.** Complete the table below for each individual who will be involved in any of the surgeries on this protocol.

Name	Surgery #(s) (see Item 1)	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted].	1,2,3,4, 5	(X)	(X)	(X)	( )
[redacted]	1,2,3,4	(X)	(X)	(X)	( )
[redacted]	1,2,3,4, 5	(X)	(X)	(X)	( )

[redacted]	1,2,3,4, 5	( )	(X)	(X)	( )
[redacted]	1,2,3,, 54	( )	(X)	(X)	( )
[redacted]	1,2,3,4, 5	( )	(X)	(X)	( )

4. **Location of surgery.** Complete the table below for each location where surgery on this protocol will be performed.

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
[redacted]		1,3, 4, 5	(X)	( )*	( )*
		2	( )	(X)	( )*
			( )	( )*	( )*
			( )	( )*	( )*

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
►

5. **Pre-operative protocol.**

   a. **Pre-operative procedures.** Complete the table below for each pre-operative procedure that will be performed to prepare the animal(s) for surgery.

Surgery #(s) (see Item 1)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1	(X) – 12 hours	( )	(X) – Front leg	( ) --
2	(X) – 12 hours	( ) --	(X) – Front leg, and Femoral artery in back leg	( ) --
3	(X) – 12 hours	( ) --	(X) – Front leg	( ) --
4	(X) -- 12 hours	( ) --	(X) – Front leg	( ) --
5	X- 12 hours		X) – Front leg	

b. **Pre-operative medications.** Complete the table below. Include agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered prior to preparation of the surgical site on the animal.

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Brevital	1,2,3,4, 5	6-10 mg/kg to effect	IV	Once	immediate
Acepromazine	1,2,3,4, 5	0.05-0.1mg/kg	IV	Once	1 hour before
Pentobarbital	1,2,3,4, 5	30mg/kg to effect	IV	Once, (if brevital not available)	immediate
penicillin	1,4	900,000 units	IM	Once	immediate
famotidine	1,4	0.5-1.0mg/kg	oral	SID	immediate
Buprenorphine	1,4	0.01-0.02 mg/kg	IM	once	immediate
Amiodarone	2	5mg/kg	IV	Once	immediate

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ► **Fur will be shaved from all surgical sites. The sites will be scrubbed with betadine and all survival procedures will be done using sterile techniques and surgical drapes.**

Surgery 2 ► **Fur will be shaved from all surgical sites. The sites will be scrubbed with betadine and all survival procedures will be done using sterile techniques and surgical drapes.**

Surgery 3 ► **Fur will be shaved from all surgical sites.**

Surgery 4 ► **Fur will be shaved from all surgical sites. The sites will be scrubbed with betadine and all survival procedures will be done using sterile techniques and surgical drapes.**
Surgery 5 ► **Fur will be shaved from all surgical sites. The sites will be scrubbed with betadine and all survival procedures will be done using sterile techniques and surgical drapes.**

6. **Intra-operative management.**

a. **Intra-operative medications.** Complete the table below for each agent that will be administered to the animal during surgery.

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
Isoflurane	No	**1,2,3,4, 5**	**1-4%**	**Inhalation**	**Continuous**
Pentobarbital	No	**1,2,3,4, 5**	**30mg/kg to effect**	**IV**	**Once, if brevital not available**
Brevital	No	**1,2,3,4, 5**	**6-10 mg/kg to effect**	**IV**	**Once**
Epinephrine	No	**1, 2,4**	Low dose (0.01 mg/kg) every 3–5 min early; high dose (0.1 mg/kg) after prolonged; 1 ml	**IV**	**if needed for VF**
Amiodarone	No	**1,2,4**	7mg/kg	**IV**	**Single dose if needed for VF**
Heparin	No	**2**	150-200 units/kg	**IV**	**Single dose**

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.
►

b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).
► Animals will be placed on a water heated pad and extremities and lower half covered with blanket during the protocols and body temperatures will be monitored via rectal probe. All animals will have venous access through which fluids can be given to prevent dehydration. Animal will continue with water heated pads during the immediate postsurgical period.

c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.
►The level of anesthesia will be continuously monitored by observation of arterial pressure and heart rate during surgical manipulations. Any increase in pressure or heart rate in response to surgical stimuli will be interpreted as indicative of inadequate anesthesia and supplemental doses will be given. Average heart rate for the dogs under anesthesia is 85-100 bpm. If heart rate increases by more than 5bpm from the average to this point, isoflurane may be temporarily increase by .5% (no more than 3% during surgery). In addition, corneal, palpebral, and toe-pinch responses will be monitored every 15 minutes and supplemental anesthesia given as needed.

7. **Survival surgery considerations.** For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a. – 7.g.

a. Complete the table below for each survival surgery listed in Item 1, above.

Surgery # (see Item 1)	Survival Period	Measures for Maintaining Sterility							
		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
1	**5-6 months**	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
2	**4 weeks**	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
4	**No more than 4 months**	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
5	**5-6 months**	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*

* Describe any "other" measures to be taken to maintain sterility during surgery.

►

b. For each surgery, describe the immediate post-operative support to be provided to the animals.

For surgeries 1, 2, 4 and 5: The PI will be present during the post-operative period. After which he will be readily available should the technicians require assistance. Once extubated, the animal will be monitored until they are 2 hours post sternal. Acceptable heart rate parameters to leave the animal once sternal are 70- 160 bpm. Acceptable blood pressure parameters to leave the animal once sternal are systolic 90-160 mm of Hg and diastolic 50- 100 mm of Hg and respiration rate of <30.

c. Post-operative analgesia. Complete the table below for each surgery listed in item 1, above.

Surgery # (see Item 1)	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1,4,	**Buprenorphine**	**0.01-0.02 mg/kg**	**IM**	**twice a day**	**1-3 days**
1,4	**Carprofen**	**2mg/kg**	**Oral**	**SID**	**1-3 days if needed**
1,2,4	**Diazepam**	**0.2-2 mg/kg**	**Oral or IM**	**BID if needed**	**PRN**
1,2,4, 5	**meloxicam**	**0.2mg/kg**	**IM or SQ**	**Once, or as alternative to Carprofen**	**1 day**

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:

►

d. Other post-operative medications. Complete the following table to describe all other medications that will be administered as part of post-operative care.

Surgery # (see Item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
1,2,4, 5	**Baytril**	**5-20mg/kg**	**Oral**	**SID (in place of Cefpodoxime)**	**10 days or as needed**
1,2,4, 5	**Cefpodoxime**	**5mg/kg**	**Oral**	**SID**	**10 days or as needed**
1,4	**bismuth subsalicylate**	**262mg**	**oral**	**SID**	**As needed**
1,4	**famotidine**	**0.5-1.0mg/kg**	**oral**	**SID**	**As needed**
1,4	**Metoclopramide**	**0.5 mg/kg**	**IV**	**SID**	**As needed**

e. Post-operative monitoring. <u>After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency</u>.

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1,2,4, 5	**Constantly**	**4-6 hours**	[redacted]

(2) Post-operative monitoring after the immediate post-operative period

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1,2,4, 5	**2 times/day for 5 days**	**20 minutes**	[redacted]

f. Post-operative consequences and complications.

(1) For each surgery, describe any common or expected post-operative consequences or complications that may arise and what will be done to address them.

Surgery 1 ► During the surgical procedure, internal bleeding may occur due to cardiac or vessel laceration (lung or cardiac vessel). The PIs have learned a technique from a cardiothoracic surgeon to repair lung lacerations but this still may not be survivable If bleeding from any location becomes uncontrollable or not repairable, the animal will be euthanized by exsanguination under anesthesia. Pneumothorax (inadequate seal of thoracotomy incision) can also occur immediately post operatively. This will require an emergency surgery to reclose the incision.
Post operatively, the major concern is pain which can be treated effectively with analgesics. Animals that remain anorexic or with reduced appetite will be given IV or SQ fluids and treated with anti-emetics (metoclopramide and famotidine)
Standard wound care will be provided. Incision infections can occur and will be treated with oral and topical antibiotics.
Surgery 2 ► Major concern is pain which can be treated with analgesics. Standard wound care will be provided. To avoid risk of bleeding from the femoral artery, after procedure is completed, hemostasis sheath will be removed over a wire and hemostasis obtained with manual pressure for at least 20-30 minutes. There are rare risks associated with myocardial biopsies such as blood clots, bleeding, abnormal heart rhythm, infection, collapsed lung, injury to the artery, and rupture of the heart. This risks will be managed by performing this procedure in a sterile environment, providing antibiotics upon any sign of infection, keeping the animal anesthetized for at least 30 minutes post-surgery, and administrating diazepam post-surgery to keep the animal calm for 24 hours. If there are any indications of these injuries during the procedures the animal will be exsanguinated or terminated via pentobarbital overdose. If there is any evidence of these injuries post procedure, the animal will be immediately scheduled for a terminal surgery.
Animal will be monitored during a recovery phase of 4 hours.

During this procedure, there is a risk to damaging the femoral nerve, which runs next to the femoral vein, where the biopsy is performed. Damage to this nerve could cause decreased sensation, numbness, burning or pain in the leg. This risk will be managed by taking great care not to touch the nerve during the procedure and to observe the dog for any dysfunction in the leg up to 7 days post-operative.

This procedure is novel to the PI and any unforeseen complications will be reported to the veterinarian immediately as well as to the IACUC committee.

Surgery 3 ►**NA**

Surgery 4 ► During the surgical procedure, internal bleeding may occur due to cardiac or vessel laceration (lung or cardiac vessel). The PIs have learned a technique from a cardiothoracic surgeon to repair lung lacerations but this still may not be survivable. If bleeding from any location becomes uncontrollable or not repairable, the animal will be euthanized by exsanguination under anesthesia. Pneumothorax (inadequate seal of

thoracotomy incision) can also occur immediately post operatively. This will require an emergency surgery to reclose the incision.
Post operatively, the major concern is pain which can be treated effectively with analgesics. Animals that remain anorexic or with reduced appetite will be given IV or SQ fluids and treated with anti-emetics (metoclopramide and famotidine)

Surgery 5-Post operative infection or intraoperative bleeding are the main concerns. If bleeding from any location becomes uncontrollable or not repairable, the animal will be euthanized by pentobarbital overdose. Post operatively, the major concern is pain which can be treated effectively with analgesics. Animals that remain anorexic or with reduced appetite will be given IV or SQ fluids and treated with anti-emetics (metoclopramide and famotidine)

(2) List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1 ► **Any animal in persistent pain or distress after 72 hours that cannot be adequately treated with analgesics will be euthanized. Signs of distress and pain will suspected in the presence of weight loss (12% of body weight), lethargy, limping, vocalizing, excessive licking for over 2-3 days and even aggression.**

Surgery 2 ► **Any animal in pain or distress that cannot be adequately treated with analgesics will be euthanized. Signs of distress and pain will suspected in the presence of weight loss (10% of body weight), lethargy, limping, vocalizing, excessive licking for over 2-3 days and even aggression. If post-operative bleeding occurs and cannot be controlled, the animal will be euthanized.**

Surgery 3 ►Terminal surgery

Surgery 4 ► **Any animal in pain or distress after 72 hours that cannot be adequately treated with analgesics will be euthanized. Signs of distress and pain will suspected in the presence of weight loss (10% of body weight), lethargy, limping, vocalizing, excessive licking for over 2-3 days and even aggression.**

Surgery 5- **The major concern for this minor procedure is pain and this will be treated with analgesics. Standard wound care will be provided. Animals who are slow to resume normal eating patterns will be given fluids either IV or SC and anti-emetics..**

(3) In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)

► **No restrictions**

g. Maintenance of post-surgical medical records. Complete the table below for each surgery, specifying where the records will held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

Surgery # (see Item 1)	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1,2,3,4, 5	[redacted]	[redacted]	(X)	( )
2			( )	( )
3			( )	( )
4			( )	( )

8. **Certification.** The PI must sign the certification statement in Item Z.5 of the main body of the ACORP.

ACORP APPENDIX 6
SPECIAL HUSBANDRY AND PROCEDURES
VERSION 4

See ACORP App. 6 Instructions, for more detailed explanations of the information requested.

1. **Description of Procedures.** Complete the table below for each procedure listed in Item V of the main body of the ACORP that is not detailed in a SOP or in another item or Appendix of the ACORP. For each special procedure, check all features that apply.

Special Procedure		Features							
Number	Brief Description	Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Conditioning	Irradiation	Imaging	Other**
1	**Nerve recording. Cage cleaning and letting of dogs out of cage will need to be done one dog at a time, so as to avoid data crosstalk between cages (receivers meant for another dog picking up data from this dog as the dog runs around the room). Also, times will have to be pre-specified so the P.I. can expect when data drop out or potential cross talk occurs.**	(X)	()	( )	( )	( )	( )	( )	( )
2		()	( )	( )	( )	( )	( )	( )	( )
3		( )	( )	( )	()	( )	( )	( )	( )
4		( )	( )	( )	( )	( )	( )	( )	( )

*Husbandry refers to all aspects of care related to the maintenance of the animals, including (but not limited to) provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

**Describe any "Other" features that are involved.
►

a. Provide a complete description of each special procedure listed above, including the duration of the procedure, how frequently it will be repeated in any one animal, and any effects it is expected to have on the animal:

Special Procedure 1 ► **Typically cage cleaning is performed daily with dogs let out of the cage in random fashion. However, because the receivers installed in each cage has the capability of picking up signals from any dog that is in close proximity to it, letting dogs out all at the same time has the ability to cause data cross talk, thus invalidating the data for that period of time when the dogs are out of the cage. For this reason, the dogs with transmitters in the same room, have to be let out individually, returned to the cage, before another dog with a transmitter be let out of its cage. The times need to be**

documented as well so the P.I. can expect when data drop out or potential cross talk occurs. Cleaning times will be posted clearly on the dog runs and communicated to the VMU supervisor and caretakers

Special Procedure 2 ►

Special Procedure 3 ►

b. Explain why each of these special procedures is necessary:

Special Procedure 1 **As soon as PVC software patch is enabled, DSI radio telemetry device will be turned ON to record PVC features (border, OrRs duration and precautions), heart rate variability and continuous blood pressure recordings. The cleaning schedule is in place so that there can be at least 24 hours of continuous data collected from the devices. The animals are removed one at a time to avoid any cross talk that could occur between different transmitters and receivers in the room.**

Special Procedure 2 ►

Special Procedure 3 ►

2. **Personnel.** Complete the table below for each special procedure listed in Item 1, above. Identify the individual(s) who will be responsible for carrying out the procedures, and those who will be responsible for monitoring the condition of the animals during and after the procedures. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

Procedure Number (see Item 1)	Responsible Individual(s)	
	Carrying Out Procedure	Monitoring the Animals
1	[redacted]	[redacted]

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so, describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

Procedure Number (see Item 1)	Expected Potential Pain and/or Distress			
	No	Yes		
		Description	To Be Relieved	Not to Be Relieved
1	(X)		()[a]	( )[b]
2	()		( )[a]	( )[b]
3	()		( )[a]	( )[b]
4	( )		( )[a]	( )[b]

a. For each procedure for which potential pain and/or distress is expected, but WILL be prevented or alleviated by administration of the analgesic(s) or stress-relieving agents, complete the table below:

Procedure Number (see Item 1)	Agent	Dose (mg/kg) & vol (ml)	Route of admin	Freq of admin (times/day)	Duration of admin (days post-procedure)
1					
2					
3					
4					

Describe any non-pharmacological measures to be taken to address the potential pain and/or distress:

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

b. For each procedure for which potential pain and/or distress is expected and will NOT be prevented or alleviated, provide the scientific justification for this:

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the special procedures, and list the criteria that will be used to determine when individual animals will be removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

Procedure Number (see Item 1)	Monitoring Methods	Endpoint Criteria
1	**Present with dog and blood pressure monitoring during procedure and close observation throughout full recovery**	**Drug challenge would not be obtained in those animals with any kind of distress / recent wound or surgical procedures.**

2		
3		
4		

Secondary Review

PI	Station	Funding Source	Application Title
[redacted]	Richmond, VA - 652	NIH	Mechanistic Insight of Premature Ventricular Contractions-induced Cardiomyopathy

Action Needed by IACUC
The IACUC must review the concerns listed below and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s) and the revised ACORP(s) must be forwarded to the CVMO for archiving.

In case of questions about this review, please contact [redacted] Assistant Chief Veterinary Medical Officer at [redacted]

Reviewer Feedback

ACORP Item number(s)	Comments/Concerns
ACORP (dog)	This ACORP uses a canine model of premature ventricular contractions (PVCs) to investigate why PVCs cause the heart to contract less effectively, which often leads to heart failure. The investigator is commended for providing a clear rationale for the timing and number of the heart biopsies, the justification of the canine PVC model, the reward-based training program related to cardiac assessment and treadmill procedures, and his presence during the post-operative period. Some aspects of protocol should be clarified. An appendix to this review provides additional information for the IACUC's consideration. The specific numbered comments provided below must be reviewed by the IACUC, to determine what responses are needed. These actions must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP and the revised ACORP provided to the CVMO for archiving.
Item C.2	In general, the investigator has made an effort to define and explain the proposed cardiovascular terms and procedures with the exception of the following: post-extrasystolic, pericardial cradle, a sawing sleeve (pacemaker implant), ventricular capture, and sledinger technique. Understanding of the study would be improved if these terms were explained. In the description of the treadmill training, the investigator states "All animals thus far appear to be class 1HF, but technically some of them may start at class 1 and later transition into class II HF. Presumably, HF refers to heart failure; please provide a description of each heart failure class.
Items C.2, J, and U	Items C.2 and U indicate a final (terminal) heart biopsy is performed and will result in euthanasia by exsanguination under anesthesia; please list this category D procedure in item J.

(cont.)

Item T and Appendix 5 (1)	The investigator indicates internal defibrillation paddles and epinephrine will be used as part of a resuscitation procedure but inadvertently substituted another word for resuscitation. Please reconcile. In item T, >12% weight loss is listed as one indicator of possible pain and distress; however in Appendix 5- item 7.f.2., weight loss as an indicator of pain and distress varies from 10% to 12%. Please explain.
Item U	If the euthanasia method is an overdose of pentobarbital, please clarify how death is confirmed or if a second physical method of euthanasia is performed.
Appendix 4	The second sentence in the response to item 2.a contains a nonsensical word, please correct**.**
Appendix 5	In item 1.a and 1.b of this appendix, the investigator states "Wound revision - it is not uncommon for surgical wound dehiscence to occur immediately after surgery. "and Wound revision- This would occur 2-7 days after the initial surgery if needed.", respectively. Wound dehiscence soon after surgery is often related to the sutures being tied too tightly, which compromises blood supply. In dogs, the sutures should be tightened (tied) just enough to appose the skin edges; the surgeon should be able to place the tip of a small hemostat beneath each interrupted suture. Skin breakdown over a subcutaneously implanted device is also usually related to pressure necrosis. The information found at the following link may be helpful: https://www.acvs.org/files/proceedings/2012/data/papers/122.pdf

Appendix - Additional Suggestions for Improvement

Comment 1: Part B. This section would be strengthened for the lay reader by starting it with the relevance of this work to human heart disease. Also, the specific relevance to Veterans' health is not specified. Try putting something like this at the beginning of the section:

The focus of our research is heart disease, specifically cardiomyopathy which the CDC website says affects as many as 1 in 500 adults in the United States (this adds up to approximately 600,000 Americans) and that long term heavy alcohol use is one cause *(https://www.cdc.gov/heartdisease/cardiomyopathy.htm accessed 10/10/17).* The VA website says sixty to eighty percent of Vietnam Veterans seeking PTSD treatment have alcohol use problems, making this group of Veterans particularly prone to cardiomyopathy *(https://www.ptsd.va.gov/public/problems/ptsd-alcohol-use.asp accessed 10/10/17).* We have found that the best way to study cardiomyopathy is with dogs, both because the dog heart anatomy is so similar to the human heart anatomy, and dogs are large enough for the pacemakers and radiotelemetry devices this work requires.

(cont.)

Comment 2: **Part B.** Technical comment: The title and the language in part B refer to "premature ventricular contractions" or PVCs, yet part B says this study is actually about premature upper chamber heart beats. Specifically, it says "**Frequent upper chamber premature heart beats will be replicated via an implantable pacemaker/defibrillator**" Shouldn't these be referred to as premature atrial contractions, or PACs rather than PVCs? If so, the title and language throughout the ACORP should be reconciled.

Comment 3: **Part D:** **The justification for the species choice could be made clearer and easier for the lay reader to understand.**

Try something like this:

For this study we need to deliver PVCs in a controlled fashion, and also have an implanted radiotelemetry device providing real-time data on the heart's electrical activity. The only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated electronic defibrillator/pacemaker specifically developed for our study. It is approximately 2 inches long, ¼ inch thick and 1.5 inch wide, making it far too large to implant into smaller species such as rabbits or guinea pigs. Although "biological pacemakers" have been developed for smaller species, they cannot be programmed and do not store or analyze data, so they are not suitable for this study. The radiotelemetry device we need to implant is also too large for smaller species.

Our only options for this work are large animals such as dogs and pigs. Dogs are much more suitable for this work because the canine heart has a His-Purkinje system located in endocardium just like in the human heart. Pigs and other larger animals have a different anatomy, so work with them would not be as relevant to the human condition. Canine heart physiology has also been extensively studied over many years so a lot of information is already available that our study builds upon. Switching to another species such as pigs would require us to start over to some degree, running a lot of pig experiments to reach the point where we already are with dogs before we could even perform this particular study. This process would use many more pigs to get to that point than the 54 dogs required for this study.

Comment 4, **section W1 table** **(literature search). This literature search would be strengthened by doing the following:**

Change how the search terms are run. Running all the potentially painful or distressing procedures together in a single search means only a paper that includes all of those search terms would be found. Run separate searches instead, since there may be papers that address individual procedures.

In the example below, the first row is a search on PubMed for unnecessary duplication, focusing on this particular study.

The second row is an ALTBIB search specifically for papers where alternatives to using animals for this kind of research is the main topic. This was done using the ALTBIB website run by NIH https://toxnet.nlm.nih.gov/altbib.html (This website works with Google Chrome, but not with Internet Explorer)

(cont.)

The rest are for potentially painful or distressing procedures, using the ALTBIB search for citations from 2000 to present. Each of these searches brings up less than 30 papers.

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
PubMed	1/8/18	1966-2018	N/A	ventricular bigeminy, cardiomyopathy	( )	( )	( )	(X)
ALTBIB Citations with Animal Use Alternatives as the main topic	1/8/18	1966-2018	N/A	ventricular bigeminy, cardiomyopathy	(X)	( )	( )	( )
PubMed using ALTBIB animal alternatives search strategy	1/8/18	2000-2018	Cardiac pacemaker implantation	Cardiac pacemaker implantation	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	1/8/18	2000-2018	percutaneous biopsy	"percutaneous myocardial biopsy"	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	1/8/18	2000-2018	Lead revision	cardiac lead revision	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	1/8/18	2000-2018	Blood draw	Blood draw, brachial vein	(X)	(X)	(X)	( )
PubMed using ALTBIB animal alternatives search strategy	1/8/18	2000-2018	Blood draw	Blood draw, jugular vein	(X)	(X)	(X)	( )

(cont.)

Comment 5, section W2: (Replacement)

This section would be strengthened by including the following information:

a) **Please explain why this research cannot be done with *in vitro* methods (it may be obvious to any biomedical scientist, but this still has to be included). You can say something like** "We ran a search specifically looking for animal use alternatives for this kind of research, and there were no in vitro, computer, or other non-animal models available."

b) **The text states that the PI recently described the first canine model of PVC-induced cardiomyopathy in canines. However, another group has described PVC-induced cardiomyopathy in pigs (** https://www.ncbi.nlm.nih.gov/pubmed/26416621 **) Please explain in detail why this work cannot be done in pigs, perhaps focusing on how pig hearts do not have the His-Purkinje system located in endocardium.**

Comment 6, section W3: (Reduction) A line can be added to the end of this part directing the reader to the detailed statistical analysis in section C2b. Try something like: "See section C2b for details."

Comment 7, section W4 (refinement): The methods used to acclimate the dogs to the treadmill are also a refinement and should be included here. Also, please include a statement referring to the literature search such as.

"We ran searches for ways to minimize pain and distress for the various procedures, and did not find any refinements over our current methods."

Comment 8, section W5 (lack of unnecessary duplication)

The answer provided in this ACORP would be strengthened by providing some more detail.

Try something like this:

Our literature search for "ventricular bigeminy, cardiomyopathy" yielded 29 papers, of which X are from our group. ***[Then explain how this study differs from or builds on these 29 papers.]***

(cont.)

Literature search 652 Richmond [redacted]

1) **How is this research relevant to Veterans health?**

This is a study on how preventricular contractions (PVCs) lead to cardiomyopathy. Two kinds of PVCs will be studied: short-coupled PVCs (coupling interval of 200-220ms) and long-coupled PVCs (coupling interval of 320ms) to see how coupling interval contributes to the development of cardiomyopathy.

The CDC website says cardiomyopathy affects as many as 1 in 500 adults in the United States (this adds up to approximately 600,000 Americans) and that long term heavy alcohol use is one cause *(https://www.cdc.gov/heartdisease/cardiomyopathy.htm accessed 3/10/18).* The VA website says sixty to eighty percent of Vietnam Veterans seeking PTSD treatment have alcohol use problems, making this group of long-suffering Veterans particularly prone to cardiomyopathy *(https://www.ptsd.va.gov/public/problems/ptsd-alcohol-use.asp accessed 3/10/18).*

2) **Is this work <u>unnecessarily</u> duplicating work already documented in the literature?**

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
PubMed	3/12/18	All available years	PVC, coupling interval, cardiomyopathy	16

A PubMed search brought up 16 papers, of which 11 were observational studies not focused on the question the current study will examine. Of the remaining 5 papers, two were from this research group, and the current study will build on those papers. Two other papers were a clinical trial comparing catheter ablation to antarrhythmic drugs, and a case report. One paper was a study in PVC and interval coupling, but it was looking at the effects of different coupling intervals on the intrinsic cardiac nervous system and repolarization, not on the development of cardiomyopathy. The current study does not duplicate any published work.

3) **Could this work be done in computer models or in vitro (tissue culture)?**

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
ALTBIB Citations with <u>Animal</u>	3/11/18	All available years	PVC, coupling interval, cardiomyopathy	0

Use Alternatives as the main topic				

An ALTBIB search for “alternatives to using animals” for this study yielded no papers at all. No computer models or in vitro models for this work were found.

4) Could it be done in non-mammals or in other mammals?

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
ALTBIB animal alternatives search strategy - all citations	3/11/18	2000-present	PVC, coupling interval, cardiomyopathy	0

An ALTBIB search for all relevant citations brought up no papers.

Small mammals such as rats, mice, guinea pigs and rabbits cannot be used for this project because the only technology available to deliver PVCs in a controlled fashion is through a special highly sophisticated (approximately 2 inches long, ¼ inch thick and 1.5 inch wide) electronic defibrillator / pacemaker that is too large to use in small animals. The radiotelemetry device is also large and will require internal implantation and observation for several months.

Dogs are used for two reasons: 1) Atrial fibrillation can be readily induced in dogs and 2) The group has been using dogs for over 25 years. In order for the new data to be comparable with the previously collected data, they need to continue to use dogs. Switching to another species would to some degree be starting over, and require many more animals than this study will use.

Although in principle pigs could be used, the electrophysiology of the pig heart differs from the human and canine heart in a significant way, specifically the endocardium and epicardium are activated simultaneously in the swine heart but not in the human or canine heart [Lelovas 2014], and this discordance in the human and dog heart can play a role in the development of cardiomyopathy. Additionally, dogs have a His-Purkinje system located in endocardium, very similar to the human heart, that pigs and other larger animals do not have [Newton 2004].

5) **Are the methods used the best available (least painful or distressing to the dogs)?**

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	How many papers were found?

ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	chronic PVC-induced left ventricular dysfunction	chronic PVC-induced left ventricular dysfunction	0
ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	thoracotomy	thoracotomy, "cardiac surgery"	5
ALTBIB animal alternatives search strategy - all citations	3/11/10	2000-2018	symptomatic congestive heart failure	"symptomatic congestive heart failure", canine	0

We ran multiple searches for better methods:

1) A search on ALTBIB for “chronic PVC-induced left ventricular dysfunction” yielded no papers.
2) A search on ALTBIB search for “thoracotomy, cardiac surgery" gave 5 papers. Two were on rats, one was one sheep, and one was on mice. The fifth paper was about transcatheter pulmonary valve replacement, which is very different from what we are doing in this study. We did not find refinements for thoracotomy in canines for our studies.
3) A search on ALTBIB for “symptomatic congestive heart failure, canine” yielded no papers.

This group has extensive experience in this surgical model of PVC-induced cardiomyopathy, a model that they designed. The method has been refined so the cardiomyopathy develops gradually without symptomatic congestive heart failure or signs of distress.

<u>The treadmill procedure is designed to minimize distress for the dogs:</u>

> Dogs allowed to them explore the exercise room and equipment until they have become comfortable with those surroundings. Presence of normal, relaxed behavior will signal that the dogs are ready for the next step, which is putting them on the treadmill while it’s turned off. This will occur in small steps, putting them on for seconds and then extending the time. Each positive reaction will be rewarded with treats to encourage the dogs’ learning process. When the dogs have become relaxed with the task of being on the still treadmill, they will next be put on the treadmill at its slowest speed, 0.5 mph. Two people will assist in this process; one person will hold the leash of the dog and stand in front of the treadmill offering rewards for positive behavior while the other will stand behind the animal making sure that it does not slide off of the machine or jump off of the sides. This person will also to help the dog move its feet until it begins to understand and be comfortable with the movement. The process will take as long as needed to have the dogs become comfortable with the treadmill**.**
>
> The treadmill workout will be done a total of 4 times in our study. The first 2 workouts will be performed 1-2 days apart at baseline about 2 weeks post-surgery after sutures have been removed. The final 2 treadmills will occur 1-2 days apart at the end of the study before final surgery.

Each workout lasts 10 minutes, in which the dogs will complete 3 stages, each lasting 3 minutes. at the first stage is at 1.1 mph followed by three minutes at 2.3 mph, and then three minutes at 3.3 mph. (Normal human walking speed is about 3 mph).

The procedures for echocardiograms, blood draws, etc., are also designed to minimize distress for the dogs:

Non-surgical procedures such as echocardiograms, electrocardiography, pacemaker interrogation and blood draws will be performed in conscious dogs with minimal or no sedation. In order to achieve this, all animals will undergo training to lay or sit down still for 20-30 minutes during the procedures. We estimate that this training will take from 2-4 weeks. Methods used for training will consist mostly of repetition with rewards as the periods of lying or sitting still are gradually extended.

However, if an animal cannot be trained to sit or lie supine for 10 minutes for the echocardiogram, we will first attempt to mildly sedate the animal with Acepromazine (0.05-0.1mg/kg) given PO approximately 1 hour prior to the procedure. If this is unsuccessful we will have to perform echocardiogram under general anesthesia with endotracheal intubation. We will use Brevital (6-10mg/kg) IV to effect (or Pentobarbital 30mg/kg, if Brevital is not available). Animals will be intubated, mechanically ventilated and anesthetized with isoflurane 1-3%. After the echocardiogram, they will be allowed to recover from anesthesia in a post-operative recovery cage until able to walk to their kennel. No analgesics will be necessary due to the non-invasive nature of this procedure.