INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.
THE ELECTROCARDIOGRAPHIC EVALUATION OF ARRHYTHMOGENIC CARDIOMYOPATHY IN BOXERS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Alan W. Spier, D.V.M.

******

The Ohio State University
2002

Dissertation Committee

Associate Professor Kathryn M. Meurs, Adviser
Professor Robert L. Hamlin
Professor William W. Muir
Professor John D. Bonagura

Approved by

Adviser
Veterinary Clinical Sciences
ABSTRACT

Arrhythmogenic cardiomyopathy in Boxers is characterized by the development of ventricular arrhythmias and sudden death. Standard diagnostics, including thoracic radiographs and echocardiography are of limited use in the evaluation of Boxers with arrhythmias. Alternative diagnostics are needed to better evaluate these patients, help determine prognosis and guide therapy.

Electrophysiology testing is used in the evaluation of arrhythmias in people. The need for anesthesia, the relative expense and risks inherent to the procedure, and its limited availability preclude its use in veterinary medicine. Inexpensive, noninvasive diagnostic tests that are associated with fewer risks would significantly improve the management of patients with ventricular arrhythmias. The goal of this project was to evaluate Boxers with arrhythmias by the use of noninvasive diagnostic testing, including ambulatory electrocardiography, signal-averaged electrocardiography, QT dispersion, and heart rate variability.

The degree of arrhythmia variability evaluated by ambulatory electrocardiography can affect disease diagnosis and influence assessment of progression and efficacy of therapy. Substantial variability was identified, and suggests that an 80% reduction in arrhythmia frequency is necessary to document a drug effect, while changes of less than 80% may be within the limits of spontaneous variability.
Signal-averaged electrocardiography documented the presence of late potentials in Boxers that were most severely affected. The presence of late potentials suggests that abnormal conduction exists in these dogs, which may represent a substrate for arrhythmia formation. Identification of late potentials may therefore be a predictor for mortality.

QT dispersion from standard electrocardiographic tracings was used to identify abnormalities in repolarization. The degree of QT dispersion did not correlate with any measure of disease severity. Many severely affected dogs had no QT dispersion, and QT dispersion was identified in some less affected dogs. QT dispersion was therefore not considered to be a useful diagnostic test in these dogs.

Analysis of heart rate variability was reduced in dogs with congestive heart failure, but no reduction was identified between affected and unaffected dogs. Increased sympathetic tone is unlikely to be a discriminating feature between unaffected Boxers and those with only arrhythmias. Therefore, analysis of heart rate variability was of little value in assessing arrhythmic disease.
Dedicated to all the Boxers that “volunteered” to be in the study,
and to all those that never had the chance.
ACKNOWLEDGMENTS

I wish to thank my advisor, Dr. Kate Meurs for her seemingly endless support and encouragement; for the many times she used carrots instead of sticks. Without her effort and enthusiasm, this project would have never been possible.

I extend my deepest appreciation, respect, loyalty and admiration to Dr. Robert Hamlin for his guidance and support. He is a role model, whose influence on me personally and professionally, are beyond words. I am truly privileged to have had the opportunity to watch him work.

I thank Drs. John Bonagura and William Muir for their service as committee members and their intellectual stimulation and constructive criticism.

I am indebted to Nicola Wright, who through the evolution of this project, became the chief organizer, secretary, dog-holder, and, at times, cheerleader.

A special note of gratitude to Dr. Craig Hassler, Jeff Wallery, and Tom Vinci at the Battelle institute for their generosity for allowing us both time and space to analyze Holters.

I would like to acknowledge The Ohio State University canine research funds and the AKC/Boxer charitable trust for their financial support.

Finally, to all the veterinary students and volunteers who gave up their Saturday mornings to help us with holding (and sometimes wrestling) dogs.
VITA

June 25, 1970 .............................................. Born—El Paso, TX

1992 .......................................................... BS Zoology, University of Texas

1993 .......................................................... BS Veterinary Science, Texas A&M University

1996 .......................................................... DVM, Texas A&M University

Kansas State University

1997-2002 ................................................ Resident, Small Animal Medicine (Cardiology)
The Ohio State University

1997-present ............................................. Doctoral Candidate, The Ohio State University

PUBLICATIONS

Research Publication:


FIELDS OF STUDY

Major Field: Veterinary Cardiology
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
</tr>
<tr>
<td>Dedication</td>
</tr>
<tr>
<td>Acknowledgments</td>
</tr>
<tr>
<td>Vita</td>
</tr>
<tr>
<td>List of Tables</td>
</tr>
<tr>
<td>List of Figures</td>
</tr>
<tr>
<td>Background and Introduction</td>
</tr>
<tr>
<td>Chapter 1—Spontaneous Variability in VPC frequency</td>
</tr>
<tr>
<td>Introduction</td>
</tr>
<tr>
<td>Materials and Methods</td>
</tr>
<tr>
<td>Inclusion criteria</td>
</tr>
<tr>
<td>24-hour ambulatory ECG</td>
</tr>
<tr>
<td>Data analysis and statistical evaluation</td>
</tr>
<tr>
<td>Results</td>
</tr>
<tr>
<td>Discussion</td>
</tr>
<tr>
<td>Footnotes</td>
</tr>
<tr>
<td>Reference List</td>
</tr>
<tr>
<td>Chapter 2—Signal-averaged electrocardiography</td>
</tr>
<tr>
<td>Introduction</td>
</tr>
<tr>
<td>Materials and Methods</td>
</tr>
<tr>
<td>Inclusion Criteria</td>
</tr>
<tr>
<td>Ambulatory Electrocardiography</td>
</tr>
<tr>
<td>Cardiac Ultrasonography</td>
</tr>
<tr>
<td>Signal-averaged Electrocardiography</td>
</tr>
<tr>
<td>Subject Categorization</td>
</tr>
<tr>
<td>Statistical Analysis</td>
</tr>
</tbody>
</table>

viii

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>51</td>
</tr>
<tr>
<td>Discussion</td>
<td>62</td>
</tr>
<tr>
<td>Footnotes</td>
<td>70</td>
</tr>
<tr>
<td>Reference List</td>
<td>71</td>
</tr>
<tr>
<td>Chapter 3—QT dispersion</td>
<td>73</td>
</tr>
<tr>
<td>Intro</td>
<td>73</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>75</td>
</tr>
<tr>
<td>Animal selection</td>
<td>75</td>
</tr>
<tr>
<td>12-lead electrocardiography</td>
<td>75</td>
</tr>
<tr>
<td>24-hour ambulatory electrocardiography</td>
<td>76</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>76</td>
</tr>
<tr>
<td>Statistical evaluation</td>
<td>77</td>
</tr>
<tr>
<td>Results</td>
<td>77</td>
</tr>
<tr>
<td>Discussion</td>
<td>80</td>
</tr>
<tr>
<td>Footnotes</td>
<td>85</td>
</tr>
<tr>
<td>Reference List</td>
<td>86</td>
</tr>
<tr>
<td>Chapter 4—Heart rate variability</td>
<td>89</td>
</tr>
<tr>
<td>Introduction</td>
<td>89</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>90</td>
</tr>
<tr>
<td>Patient Selection</td>
<td>90</td>
</tr>
<tr>
<td>Subject Categorization</td>
<td>90</td>
</tr>
<tr>
<td>Ambulatory electrocardiography</td>
<td>91</td>
</tr>
<tr>
<td>Heart rate variability analysis</td>
<td>91</td>
</tr>
<tr>
<td>Statistical evaluation</td>
<td>92</td>
</tr>
<tr>
<td>Results</td>
<td>92</td>
</tr>
<tr>
<td>Discussion</td>
<td>100</td>
</tr>
<tr>
<td>Footnotes</td>
<td>104</td>
</tr>
<tr>
<td>Reference List</td>
<td>105</td>
</tr>
<tr>
<td>Summary and Conclusions</td>
<td>108</td>
</tr>
<tr>
<td>Bibliography</td>
<td>113</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>12</td>
</tr>
<tr>
<td>1.2</td>
<td>14</td>
</tr>
<tr>
<td>1.3</td>
<td>16</td>
</tr>
<tr>
<td>1.4</td>
<td>18</td>
</tr>
<tr>
<td>1.5</td>
<td>20</td>
</tr>
<tr>
<td>1.6</td>
<td>22</td>
</tr>
<tr>
<td>1.7</td>
<td>24</td>
</tr>
<tr>
<td>1.8</td>
<td>26</td>
</tr>
<tr>
<td>1.9</td>
<td>28</td>
</tr>
<tr>
<td>1.10</td>
<td>30</td>
</tr>
<tr>
<td>1.11</td>
<td>32</td>
</tr>
<tr>
<td>1.12</td>
<td>33</td>
</tr>
<tr>
<td>1.13</td>
<td>34</td>
</tr>
<tr>
<td>2.1</td>
<td>56</td>
</tr>
<tr>
<td>2.2</td>
<td>56</td>
</tr>
<tr>
<td>2.3</td>
<td>57</td>
</tr>
<tr>
<td>2.4</td>
<td>57</td>
</tr>
<tr>
<td>2.5</td>
<td>58</td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Holter placement</td>
<td>11</td>
</tr>
<tr>
<td>1.2 Dog 1 VPCs by day of recording</td>
<td>13</td>
</tr>
<tr>
<td>1.3 Dog 2 VPCs by day of recording</td>
<td>15</td>
</tr>
<tr>
<td>1.4 Dog 3 VPCs by day of recording</td>
<td>17</td>
</tr>
<tr>
<td>1.5 Dog 4 VPCs by day of recording</td>
<td>19</td>
</tr>
<tr>
<td>1.6 Dog 6 VPCs by day of recording</td>
<td>21</td>
</tr>
<tr>
<td>1.7 Dog 7 VPCs by day of recording</td>
<td>23</td>
</tr>
<tr>
<td>1.8 Dog 8 VPCs by day of recording</td>
<td>25</td>
</tr>
<tr>
<td>1.9 Dog 9 VPCs by day of recording</td>
<td>27</td>
</tr>
<tr>
<td>1.10 Dog 10 VPCs by day of recording</td>
<td>29</td>
</tr>
<tr>
<td>1.11 Dog 11 VPCs by day of recording</td>
<td>31</td>
</tr>
<tr>
<td>1.12 Holter hook-up</td>
<td>54</td>
</tr>
<tr>
<td>2.2 SAECG sketch</td>
<td>55</td>
</tr>
<tr>
<td>2.3 Normal vs abnormal SAECG</td>
<td>61</td>
</tr>
<tr>
<td>4.1 Holter figure</td>
<td>94</td>
</tr>
<tr>
<td>4.2 Mean RR interval significance</td>
<td>96</td>
</tr>
<tr>
<td>4.3 SDNN data and significance</td>
<td>97</td>
</tr>
<tr>
<td>4.4 ASDNN data and significance</td>
<td>98</td>
</tr>
<tr>
<td>4.5 SDANN data and significance</td>
<td>99</td>
</tr>
</tbody>
</table>
BACKGROUND AND INTRODUCTION

Ventricular arrhythmias are commonly encountered in veterinary medicine, and can be associated with a variety of systemic and metabolic conditions. However, the majority of significant, often life-threatening, arrhythmias are identified in association with underlying cardiac disease, either as the chief manifestation of the disease, or as a complicating factor. Cardiomyopathy in dogs manifests primarily as either a structural abnormality associated with poor contractile function and congestive heart failure, or by an electrical instability that predisposes to arrhythmias and sudden death.

Arrhythmogenic cardiomyopathy, or familial ventricular arrhythmias, in Boxers is a primary heart disease characterized by the development of ventricular arrhythmias and increased risk of sudden death. The disease, as it was originally described, is subdivided into three "types". Type I disease is defined by the presence of ventricular (and occasionally atrial) arrhythmias in an asymptomatic dog, whereas Type II disease is characterized by either syncope or sudden death. The majority of affected individuals have preserved systolic function, making congestive heart failure an uncommon sequelae. However, a subset of dogs does present with evidence of myocardial failure and congestive heart failure, similar to overt dilated cardiomyopathy. This so-called type III disease occurs less commonly, and is not a typical presentation for the disease. For this reason, standard diagnostics performed in most cardiac patients (such as
Thoracic radiographs and echocardiography) are of limited use in most dogs. Alternative diagnostics are needed to better evaluate these patients, and help determine prognosis and guide therapy.

In human medicine, the definitive test used in the evaluation of arrhythmias is the electrophysiology study. These types of studies are not commonly performed in veterinary medicine. The need for anesthesia, the relative expense and risks inherent to the procedure, and its limited availability preclude the use of routine electrophysiologic testing. The availability of noninvasive diagnostic tests that are inexpensive and associated with fewer risks would significantly improve the ability of clinicians to manage patients with ventricular arrhythmias.

The goal of this project was to evaluate the use of noninvasive diagnostic tests in the assessment of ventricular arrhythmias associated with arrhythmogenic cardiomyopathy in Boxers. These tests include those that are currently being used such as ambulatory electrocardiographic (or Holter) monitoring, and those that are routinely used in human medicine, but have had only limited application in veterinary medicine. These latter diagnostics include signal-averaged electrocardiography, evaluation of QT dispersion from standard electrocardiograms, and analysis of heart rate variability.

Holter monitoring is extensively used in veterinary medicine for a wide variety of conditions, including determining the cause of syncope or syncopal-like episodes, documenting the presence of arrhythmias, assessing severity of disease, and monitoring the effect of therapy. At this time, the diagnosis of arrhythmogenic cardiomyopathy in Boxers is largely performed by electrocardiography. Because of the relative insensitivity of standard in-hospital electrocardiograms, most veterinary clinicians
advocate the use of ambulatory monitoring. Unfortunately, there is no clear consensus as to what constitutes an affected animal based on arrhythmia frequency, and even less agreement regarding the initiation of therapy in an asymptomatic animal. The identification of ventricular ectopia by Holter evaluation in symptomatic animals usually warrants therapy, and many asymptomatic dogs that manifest “frequent” arrhythmias are also placed on antiarrhythmic therapy. Therapy is then assessed with follow-up ambulatory electrocardiography to document efficacy and to monitor for proarrhythmia. The assessment of both efficacy and proarrhythmia must take into account the degree of inherent variability of arrhythmia frequency. There are no studies in dogs that describe the variability of arrhythmia frequency; consequently evaluating the effect of therapy is a challenge. The initial study was performed to identify to what degree the frequency of arrhythmia varies for a particular animal evaluated by ambulatory electrocardiography.

Signal-averaged electrocardiography is a high-resolution electrocardiogram that identifies the presence of late potentials in the terminal portions of the QRS complex. The presence of late potentials suggests that a conduction abnormality exists, which may serve as a substrate for reentry, and predispose to arrhythmia formation. Signal-averaged electrocardiography has been used in veterinary medicine in the evaluation of dilated cardiomyopathy in Doberman pinschers, but its use has not been reported in Boxers.

In addition to altered conduction, abnormal repolarization can also predispose to reentry, and facilitate arrhythmia formation. Repolarization can be directly evaluated with invasive electrophysiologic testing, but can also be evaluated from the surface.
electrocardiogram. The assessment of changes in the duration of the QT interval between different leads is termed QT dispersion. QT dispersion has been used in several disease states in people as a marker for adverse arrhythmic events. Its clinical utility, however, has not been investigated in veterinary medicine. The identification of altered repolarization in Boxers may be useful in identifying at-risk patients.

The association between cardiac disease and the autonomic nervous system is well known. Patients that suffer from significant heart disease, particularly congestive heart failure, have altered autonomic balance, with an increase in sympathetic tone and a withdrawal of parasympathetic tone. The manifestation of ventricular arrhythmias is heavily influenced by the sympathetic nervous system, and excessive sympathetic tone can create or exacerbate malignant arrhythmias. An indirect measure of autonomic balance is the assessment of the inherent irregularity of heart rate. Reduction in this irregularity suggests a loss of normal autonomic modulation of the heart, which may be associated with increased sympathetic tone and/or a decrease in parasympathetic tone. The effect of this unbalance, in some diseases, correlates with mortality and cardiac death. Analysis of heart rate variability has been evaluated in Doberman pinschers with dilated cardiomyopathy, but has not been evaluated in Boxers with arrhythmogenic cardiomyopathy. Abnormally reduced heart rate variability may suggest an increase in underlying sympathetic tone, which may be important in arrhythmia formation, treatment, and assessment of risk in these dogs.
CHAPTER 1

SPONTANEOUS VARIABILITY IN VPC FREQUENCY

Introduction

Ambulatory electrocardiographic (AECG, Holter) monitoring plays an important role in veterinary medicine. Such evaluations are used in the diagnosis of disease, assessment of disease severity, evaluation of the response to antiarrhythmic therapy, and in many cases monitoring the progression of disease. Although many parameters are measured on an AECG, the number and complexity of ventricular arrhythmias are most widely used. The 24-hour ambulatory evaluation is believed, and has been recently demonstrated, to be a more sensitive indicator of an individual’s overall arrhythmic state. Furthermore, most reports in veterinary medicine use only a single recording on which to base clinical decisions. It is unknown, however, to what degree the frequency of ventricular arrhythmias varies on a day-to-day basis. Unlike human medicine, very little, if any, information concerning the biologic variability of electrical activity in dogs has been reported. Biologic variability may affect decisions in disease diagnosis, disease severity assessment, and therapeutic intervention.

Boxer dogs with arrhythmogenic cardiomyopathy manifest ventricular arrhythmias and are at risk for sudden death. Unlike other forms of cardiomyopathy,
Echocardiography is of limited use because myocardial function is typically preserved; consequently, the disease in this breed is frequently diagnosed by Holter monitoring. Based on these recordings, many of these dogs are treated with long-term antiarrhythmic therapy, and efficacy of that therapy is assessed by performing follow-up Holter analysis. The objective of this study, therefore, is to document and describe the degree of spontaneous variability in the frequency of ventricular arrhythmias in Boxer dogs affected with arrhythmogenic cardiomyopathy, and assess what implications it may have on the diagnosis and treatment of the disease. In addition, differences in both the day of recording and day of the week were evaluated to identify what effect, if any, these factors may have in the frequency of ventricular arrhythmias in these dogs.

Materials and Methods

Inclusion criteria

Boxer dogs were prospectively recruited for evaluation of 24-hour ambulatory electrocardiograms (AECG, Holter monitoring) over seven consecutive days. Dogs were included if they were free of structural heart disease based on previous echocardiographic and Doppler evaluation (including acquired myocardial and valvular disease and congenital valvular disease) and had a prior Holter recording with at least 500 VPCs per 24 hour period. The Holter recording used for study inclusion was not included in data analysis. Only those recordings at least 20 hours in duration were included, and dogs were excluded if less than 6 days of recordings were available. Only dogs over 2 years of age were considered for inclusion.
24-hour ambulatory ECG

Twenty-four hour ambulatory electrocardiograms were obtained using a seven lead, three channel electrode system, and acquired using an analog tape recorder. Electrodes were so as to approximate the frontal leads I, II, and III (Figure 1.1). Recordings were analyzed using a prospective software analysis algorithm with continuous user interaction by a trained cardiology research assistant under the guidance of a veterinary cardiologist. The following data were obtained for each 24-hour Holter recording: duration of recording, mean heart rate (HR\text{mean}), minimum heart rate (HR\text{min}), maximum heart rate (HR\text{max}), number of VPCs per recording (VPC\#), day of recording (DOR, numbered one to seven), day of week (DOW, Monday through Sunday), total number of beats, and grade of arrhythmia. If VPCs were identified, the arrhythmia was graded on the basis of a modification of the Lown grading scheme as follows: grade 1 = single, uniform VPC only; grade 2 = bigeminy, trigeminy, or multiform VPCs; grade 3 = presence of couplets or triplets, and grade 4 = RonT phenomenon or ventricular tachycardia (four or more consecutive VPCs). Animals in which no VPCs were identified were classified as grade 0.

Data analysis and statistical evaluation

Variability was evaluated for on a day-to-day basis for each dog. Additionally, differences in the day of recording (e.g. day 1-7) and day of the week (Monday, Tuesday, Wednesday, etc) were also analyzed. For dogs in which only six recordings were available over a seven day period, data points for both day of recording and day of week analysis were missing. For dogs in which recordings were taken over an eight-
day period, two recordings were obtained on the same day of the week (due to a missing
day). When evaluating for differences in VPC# relative to day of the week, a mean
value from each of the two recordings was calculated, and that combined data point for
that particular weekday was used in the statistical analysis. This created a missing data
point for day of week analysis (due to the missing day), but did not affect the day of
recording analysis because a total of seven days were obtained.

Statistical evaluation included Repeated Measure One Way Analysis of
Variance (RM-ANOVA) to identify significant differences in the variability of VPC #
among dogs relative to DOR and DOW. The non-parametric RM-ANOVA on ranks
was used because the data were not normally distributed, despite multiple transforms to
normalize the data. The presence of the missing data points (for both day of recording
and day of the week) did not allow simultaneous evaluation and assessment of
interaction between DOW and DOR; consequently two independent RM-ANOVA on
ranks were performed. Significance was defined as $\alpha \leq 0.05$.

Evaluation of day-to-day variability for each dog was evaluated by calculating
the percent difference between the maximum value and minimum value obtained during
the week. A percent difference was also obtained between the median value of the
week, and the minimum or maximum value of the week (whichever yielded the greater
value).
Results

Eleven dogs were initially evaluated by seven-day ambulatory monitoring. However, one dog was excluded because more than one 24-hour Holter recording was less than 20 hours in duration. Consequently, a total of ten dogs met the inclusion criteria. Due to either technical difficulties or client compliance issues, seven consecutive days were obtained in only six of ten dogs; six recordings were obtained over a seven day period in two dogs, and seven recordings were obtained over an eight day period in two dogs. The maximum, minimum and mean heart rate, number of VPCs per 24-hour recording, grade of arrhythmia, day of recording, and day of the week for each dog are presented in Tables 1.1-1.10. Those recordings that were missing for dogs in which only six recordings were included (both DOW and DOR), or missing days for dogs that completed all seven recordings over an eight day period (DOW only), are represented by “---” in the table. For dogs in which seven days were recorded, but were obtained over an eight day period, the weekday that was duplicated and subsequently averaged is qualified by an “*”. For each dog, the number of VPCs per recording, the mean, median, 25th and 75th percentiles, and calculations of the percent change in the number of VPCs between the minimum and maximum values during the week, as well as a percent change between the median and either the minimum or maximum values during the week (whichever yielded the greater percent change) are shown in Table 1.11.

Composite data for all dogs for day of recording (DOR) and day of the week (DOW) are shown in Tables 1.12 and 1.13, respectively. With regard to day of recording analysis (e.g. day 1-7), no difference between each day of recording was
identified. In other words, day 1 was not different relative to day 2, etc. For day of week analysis (e.g. Monday, Tuesday, etc), no difference was identified relative to the days of the week. Graphs representing the variation of VPC# for each dog are presented in Figures 1.2-1.11.
Figure 1.1. Position of electrodes for Holter placement as seen from both the left and right sides. There are 7 electrodes; 3 pair to create 3 bipolar leads (or channels), and 1 to serve as ground. Channel 1 consists of the red electrode as positive (+) and the white electrode as negative (-), approximating lead II. Channel 2 consists of the brown electrode as positive (+) and the black electrode as negative (-), approximating lead III. Channel 3 consists of the orange electrode as positive (+) and the blue electrode as negative (-), approximating lead I. The green electrode serves as ground.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Wednesday</td>
<td>2</td>
<td>45</td>
<td>207</td>
<td>68</td>
<td>62</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Thursday</td>
<td>3</td>
<td>45</td>
<td>192</td>
<td>66</td>
<td>97</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>Friday</td>
<td>3</td>
<td>52</td>
<td>146</td>
<td>73</td>
<td>83</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>Saturday</td>
<td>2</td>
<td>45</td>
<td>191</td>
<td>69</td>
<td>125</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>Sunday</td>
<td>3</td>
<td>45</td>
<td>196</td>
<td>66</td>
<td>144</td>
</tr>
<tr>
<td>1</td>
<td>---</td>
<td>Monday</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>Tuesday</td>
<td>2</td>
<td>47</td>
<td>222</td>
<td>71</td>
<td>79</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>Wednesday</td>
<td>2</td>
<td>46</td>
<td>237</td>
<td>73</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 1.1. Holter data for Dog #1. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented. In this particular dog, 7 days of recording were obtained over an eight day period. There is no data for Monday (denoted by "---"), and there are two data points for Wednesday. In subsequent day of week analysis (DOW), the values for these two data points are averaged, and the mean value is used.
Figure 1.2. The number of VPCs per 24 hour recording (VPC#) are presented for dog #1.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>Thursday</td>
<td>2</td>
<td>42</td>
<td>186</td>
<td>73</td>
<td>228</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Friday</td>
<td>2</td>
<td>41</td>
<td>175</td>
<td>69</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Saturday</td>
<td>2</td>
<td>43</td>
<td>162</td>
<td>70</td>
<td>121</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Sunday</td>
<td>2</td>
<td>44</td>
<td>159</td>
<td>71</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Monday</td>
<td>3</td>
<td>44</td>
<td>178</td>
<td>71</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>Tuesday</td>
<td>2</td>
<td>39</td>
<td>159</td>
<td>66</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>Wednesday</td>
<td>2</td>
<td>43</td>
<td>155</td>
<td>65</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 1.2. Holter data for Dog #2. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented.
Figure 1.3. The number of VPCs per 24 hour recording (VPC#) are presented for dog #2.
Table 1.3. Holter data for Dog #3. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented.

<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>Wednesday</td>
<td>0</td>
<td>39</td>
<td>144</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Thursday</td>
<td>1</td>
<td>41</td>
<td>170</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Friday</td>
<td>1</td>
<td>46</td>
<td>154</td>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>Saturday</td>
<td>2</td>
<td>38</td>
<td>134</td>
<td>67</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>Sunday</td>
<td>1</td>
<td>42</td>
<td>154</td>
<td>77</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>Monday</td>
<td>2</td>
<td>40</td>
<td>159</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Tuesday</td>
<td>1</td>
<td>45</td>
<td>141</td>
<td>68</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 1.4. The number of VPCs per 24 hour recording (VPC#) are presented for dog #3.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>Sunday</td>
<td>3</td>
<td>86</td>
<td>193</td>
<td>129</td>
<td>1951</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Monday</td>
<td>3</td>
<td>86</td>
<td>187</td>
<td>128</td>
<td>1257</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Tuesday</td>
<td>3</td>
<td>72</td>
<td>185</td>
<td>114</td>
<td>1542</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Wednesday</td>
<td>3</td>
<td>82</td>
<td>201</td>
<td>115</td>
<td>1599</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Thursday</td>
<td>3</td>
<td>86</td>
<td>196</td>
<td>116</td>
<td>1608</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>Friday</td>
<td>3</td>
<td>81</td>
<td>198</td>
<td>128</td>
<td>1988</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>Saturday</td>
<td>3</td>
<td>79</td>
<td>186</td>
<td>120</td>
<td>2325</td>
</tr>
</tbody>
</table>

Table 1.4. Holter data for Dog #4. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented.
Figure 1.5. The number of VPCs per 24 hour recording (VPC#) are presented for dog #4.
Table 1.5. Holter data for Dog #6. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented.

<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1</td>
<td>Friday</td>
<td>3</td>
<td>42</td>
<td>144</td>
<td>64</td>
<td>1336</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>Saturday</td>
<td>3</td>
<td>40</td>
<td>162</td>
<td>65</td>
<td>1609</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>Sunday</td>
<td>2</td>
<td>40</td>
<td>164</td>
<td>67</td>
<td>817</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>Monday</td>
<td>2</td>
<td>39</td>
<td>140</td>
<td>67</td>
<td>905</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>Tuesday</td>
<td>2</td>
<td>44</td>
<td>149</td>
<td>68</td>
<td>728</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Wednesday</td>
<td>2</td>
<td>42</td>
<td>158</td>
<td>73</td>
<td>702</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>Thursday</td>
<td>2</td>
<td>42</td>
<td>165</td>
<td>70</td>
<td>623</td>
</tr>
</tbody>
</table>
Figure 1.6. The number of VPCs per 24 hour recording (VPC#) are presented for dog #6.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1</td>
<td>Wednesday</td>
<td>3</td>
<td>60</td>
<td>166</td>
<td>89</td>
<td>4826</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>Thursday</td>
<td>3</td>
<td>56</td>
<td>183</td>
<td>94</td>
<td>5552</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>Friday</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>Saturday</td>
<td>3</td>
<td>75</td>
<td>161</td>
<td>111</td>
<td>20679</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>Sunday</td>
<td>2</td>
<td>62</td>
<td>174</td>
<td>93</td>
<td>5152</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>Monday</td>
<td>3</td>
<td>61</td>
<td>183</td>
<td>92</td>
<td>4447</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Tuesday</td>
<td>3</td>
<td>59</td>
<td>169</td>
<td>86</td>
<td>4500</td>
</tr>
</tbody>
</table>

Table 1.6. Holter data for Dog #7. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented. In this particular dog, 6 days of recording were obtained over a 7 day period. There is no data for Friday, which is denoted by “---".
Figure 1.7. The number of VPCs per 24 hour recording (VPC#) are presented for dog #7. There is a missing data point for recording day #3.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1</td>
<td>Sunday</td>
<td>2</td>
<td>39</td>
<td>150</td>
<td>62</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>Monday</td>
<td>3</td>
<td>40</td>
<td>169</td>
<td>65</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>Tuesday</td>
<td>2</td>
<td>38</td>
<td>154</td>
<td>61</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>Wednesday</td>
<td>2</td>
<td>40</td>
<td>192</td>
<td>66</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>Thursday</td>
<td>1</td>
<td>40</td>
<td>149</td>
<td>63</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Friday</td>
<td>2</td>
<td>43</td>
<td>154</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>Saturday</td>
<td>1</td>
<td>40</td>
<td>151</td>
<td>61</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1.7. Holter data for Dog #8. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented.
Figure 1.8. The number of VPCs per 24 hour recording (VPC#) are presented for dog #8.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1</td>
<td>Tuesday</td>
<td>3</td>
<td>38</td>
<td>101</td>
<td>58</td>
<td>9095</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>Wednesday</td>
<td>3</td>
<td>39</td>
<td>107</td>
<td>55</td>
<td>2644</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>Thursday</td>
<td>3</td>
<td>38</td>
<td>111</td>
<td>59</td>
<td>11215</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>Friday</td>
<td>3</td>
<td>37</td>
<td>108</td>
<td>60</td>
<td>6522</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>Saturday</td>
<td>3</td>
<td>41</td>
<td>114</td>
<td>63</td>
<td>10623</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>Sunday</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>Monday</td>
<td>3</td>
<td>43</td>
<td>115</td>
<td>64</td>
<td>4390</td>
</tr>
</tbody>
</table>

Table 1.8. Holter data for Dog #9. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented. In this particular dog, 6 days of recording were obtained over a 7 day period. There is no data for Sunday, denoted by "---".
Figure 1.9. The number of VPCs per 24-hour recording (VPC#) are presented for dog #9. There is a missing data point for recording day #6.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day of recording</th>
<th>Day of week</th>
<th>Grade</th>
<th>Min HR</th>
<th>Max HR</th>
<th>Mean HR</th>
<th>VPC#</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>Saturday</td>
<td>3</td>
<td>64</td>
<td>187</td>
<td>105</td>
<td>3795</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>Sunday</td>
<td>3</td>
<td>55</td>
<td>173</td>
<td>96</td>
<td>2125</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>Monday</td>
<td>3</td>
<td>50</td>
<td>184</td>
<td>94</td>
<td>1384</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>Tuesday</td>
<td>3</td>
<td>62</td>
<td>176</td>
<td>95</td>
<td>2751</td>
</tr>
<tr>
<td>10</td>
<td>---</td>
<td>Wednesday</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>Thursday</td>
<td>3</td>
<td>59</td>
<td>174</td>
<td>96</td>
<td>3831</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>Friday</td>
<td>3</td>
<td>60</td>
<td>190</td>
<td>99</td>
<td>5103</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>Saturday</td>
<td>3</td>
<td>67</td>
<td>186</td>
<td>106</td>
<td>6194</td>
</tr>
</tbody>
</table>

Table 1.9. Holter data for Dog #10. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented. In this particular dog, 7 days of recording were obtained over an eight day period. There is no data for Wednesday (denoted by "---"), and there are two data points for Saturday. In subsequent day of week analysis (DOW), the values for these two data points are averaged, and the mean value is used.
Figure 1.10. The number of VPCs per 24-hour recording (VPC#) are presented for dog #10.
Table 1.10. Holter data for Dog #11. Day of recording, the day of the week, grade of arrhythmia, minimum heart rate (Min HR), maximum heart rate (Max HR), mean heart rate (Mean HR), and total number of VPCs (VPC#) are presented.
Figure 1.11. The number of VPCs per 24 hour recording (VPC#) are presented for dog #11.
Table 1.11. Descriptive data including minimum (Min) heart rate, maximum (Max) heart rate, and median heart rate for 10 Boxer dogs. The percent difference between the maximum number of VPCs per recording and minimum number of VPCs per recording (% Diff min/max) was calculated for each dog. The percent difference between the median value for the number of VPCs during the week and either the minimum or maximum number of VPCs was also calculated; whichever yielded the greater percent change is reflected in the table.
<table>
<thead>
<tr>
<th>Dog#</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Mean</th>
<th>Median</th>
<th>75th %</th>
<th>25th %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>62</td>
<td>97</td>
<td>83</td>
<td>125</td>
<td>144</td>
<td>79</td>
<td>90</td>
<td>97.1</td>
<td>90.0</td>
<td>111.0</td>
<td>81.0</td>
</tr>
<tr>
<td>Dog 2</td>
<td>228</td>
<td>44</td>
<td>121</td>
<td>20</td>
<td>35</td>
<td>48</td>
<td>33</td>
<td>75.6</td>
<td>44.0</td>
<td>84.5</td>
<td>34.0</td>
</tr>
<tr>
<td>Dog 3</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>27</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>6.3</td>
<td>2.0</td>
<td>6.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Dog 4</td>
<td>1951</td>
<td>1257</td>
<td>1542</td>
<td>1599</td>
<td>1608</td>
<td>1988</td>
<td>2325</td>
<td>1752.9</td>
<td>1608.0</td>
<td>1969.5</td>
<td>1570.5</td>
</tr>
<tr>
<td>Dog 5</td>
<td>1336</td>
<td>1609</td>
<td>817</td>
<td>905</td>
<td>728</td>
<td>702</td>
<td>623</td>
<td>960.0</td>
<td>817.0</td>
<td>1120.5</td>
<td>715.0</td>
</tr>
<tr>
<td>Dog 6</td>
<td>4826</td>
<td>5552</td>
<td>---</td>
<td>20679</td>
<td>5152</td>
<td>4447</td>
<td>4500</td>
<td>7526.0</td>
<td>4989.0</td>
<td>5452.0</td>
<td>4581.5</td>
</tr>
<tr>
<td>Dog 7</td>
<td>9</td>
<td>25</td>
<td>32</td>
<td>17</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>14.3</td>
<td>9.0</td>
<td>21.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Dog 8</td>
<td>9</td>
<td>2644</td>
<td>11215</td>
<td>6522</td>
<td>10623</td>
<td>---</td>
<td>4390</td>
<td>7414.8</td>
<td>7808.5</td>
<td>10241.0</td>
<td>4923.0</td>
</tr>
<tr>
<td>Dog 9</td>
<td>3795</td>
<td>2125</td>
<td>1384</td>
<td>2751</td>
<td>3831</td>
<td>5103</td>
<td>6194</td>
<td>3597.6</td>
<td>3795.0</td>
<td>4467.0</td>
<td>2438.0</td>
</tr>
<tr>
<td>Dog 10</td>
<td>5507</td>
<td>4730</td>
<td>2037</td>
<td>1946</td>
<td>2670</td>
<td>2243</td>
<td>1316</td>
<td>2921.3</td>
<td>2243.0</td>
<td>3700.0</td>
<td>1991.5</td>
</tr>
</tbody>
</table>

Table 1.12. The number of VPCs for each day of recording are presented for all dogs. For those dogs in which only 6 days were obtained (dogs 7 and 9), missing days are represented by "---". Mean, median, 25\text{th} and 75\text{th} percentiles are shown for each dog and for each day of recording.
Table 1.13. The number of VPCs for each day of the week is presented. For dogs in which only 6 days were obtained (dogs 7 and 9), missing days are denoted by "---". For dogs in which 7 days were obtained over an 8 day period (dogs 1 and 10), missing days are also represented by "---". In these dogs, 2 recordings were obtained on the same weekday (Wednesday for dog 1 and Saturday for dog 10). In these cases, the number of VPCs for those particular days were averaged, and the mean value is represented by the "**". 

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>---</td>
<td>79</td>
<td>76*</td>
<td>97</td>
<td>83</td>
<td>125</td>
<td>144</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>48</td>
<td>33</td>
<td>228</td>
<td>44</td>
<td>121</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>1257</td>
<td>1542</td>
<td>1599</td>
<td>1608</td>
<td>1988</td>
<td>2325</td>
<td>1951</td>
</tr>
<tr>
<td>6</td>
<td>905</td>
<td>728</td>
<td>702</td>
<td>623</td>
<td>1336</td>
<td>1609</td>
<td>817</td>
</tr>
<tr>
<td>7</td>
<td>4447</td>
<td>4500</td>
<td>4826</td>
<td>5552</td>
<td>---</td>
<td>20679</td>
<td>5152</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>32</td>
<td>17</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>4390</td>
<td>9095</td>
<td>2644</td>
<td>11215</td>
<td>6522</td>
<td>10623</td>
<td>---</td>
</tr>
<tr>
<td>10</td>
<td>1384</td>
<td>2751</td>
<td>---</td>
<td>3831</td>
<td>5103</td>
<td>4995*</td>
<td>2125</td>
</tr>
<tr>
<td>11</td>
<td>1316</td>
<td>5507</td>
<td>4730</td>
<td>2037</td>
<td>1946</td>
<td>2670</td>
<td>2243</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>1529.0</th>
<th>2428.3</th>
<th>1818.9</th>
<th>2519.5</th>
<th>1892.7</th>
<th>4242.8</th>
<th>1385.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>1257.0</td>
<td>1135.0</td>
<td>1150.5</td>
<td>1115.5</td>
<td>1336.0</td>
<td>1609.0</td>
<td>810.0</td>
<td></td>
</tr>
<tr>
<td>75th %</td>
<td>1384.0</td>
<td>4062.8</td>
<td>3165.5</td>
<td>3382.5</td>
<td>1988.0</td>
<td>2670.0</td>
<td>2125.0</td>
<td></td>
</tr>
<tr>
<td>25th %</td>
<td>35.0</td>
<td>116.8</td>
<td>29.0</td>
<td>129.8</td>
<td>144.0</td>
<td>121.0</td>
<td>20.0</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The results of this study indicate that the degree of variability is inversely related to the frequency of VPCs, with higher VPC frequency (>500 VPCs/24 hrs) associated with variability as high as 80%. This translates into a 5-fold difference between the minimum and maximum values obtained over the period of a week. Therefore, changes in VPC frequency of less than 80%, or less than a 5-fold difference, may be within the limits of physiologic variability.

By contrast, the grade of arrhythmia did not vary to the same degree. In 8 of the 10 dogs, the grade of arrhythmia only differed by one category, and in 3 did not differ at all during the week. All the dogs that did not exhibit any variability in grade had very frequent VPCs and a grade of 3 each day (the highest grade observed in the study). In only 2 dogs was there a difference of greater than one grade category within the week; this finding was observed in dogs with the least frequent arrhythmias—one with no more than 32 VPCs in any given day, and the other in the dog with the fewest number of VPCs, including zero. Therefore, the grade of arrhythmia, as defined in this study, appears less variable than the frequency of premature complexes, but appears to also vary inversely with VPC frequency.

Statistical evaluation of potential differences relative the day of recording and day of the week suggest that no predictable trends exist for either scenario. It was hypothesized that during the initial days of recording, dogs would be more agitated by the monitors, which might be reflected by an increase in the frequency of arrhythmia; as the week progressed, the dogs would become more adapted to wearing the monitors, and the VPC frequency would decrease. However, no such consistent relationship

35
between VPC frequency and recording day was observed. It was also postulated that
the day of the week may have influence on arrhythmia frequency; the owner’s activity
might vary between days (for example weekend versus weekday), which could
indirectly affect the dog’s activity and subsequently affect the number of VPCs. Again,
however, no relationship between day of the week and number of VPCs was observed.

These findings are consistent with results of similar human studies evaluating
the variability of ventricular arrhythmias. In the late 1970’s, it was observed that people
with VPCs exhibited significant variability in the frequency of these abnormal beats.
Analysis using three consecutive 24-hour AECG recordings suggested that changes in
VPC frequency independent of therapeutic intervention could account for as much as
an 83% reduction in VPC frequency. Antiarrhythmic trials evaluating variability of
VPC frequency suggested that a reduction in VPC frequency between 80-90% may be
necessary to document drug response. Concern that variability in VPC frequency
could mimic the effects of antiarrhythmic therapy led to a trial conducted in a manner
similar to antiarrhythmic drug trials, except that no medication was administered.
Using the accepted standard criterion for that time of 50% reduction in the number of
VPCs to document antiarrhythmic response, 65% of individuals would have been
determined “drug responders” had they received medication.

Few reports describing the frequency of ventricular arrhythmias in dogs are
available. Early reports suggest that the occurrence of arrhythmias in clinically normal
dogs is rare. Recent investigation into the electrical activity in normal dogs suggests
that clinically normal dogs do not possess the same degree of ectopic ventricular
activity that is seen in clinically normal people. In a study of 228 clinically normal

36

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Beagles, only 49 (21.5%) of the dogs had VPCs, and only four of these (3.125%) had more than nine VPCs in 24 hours. In a group of 49 dogs of varying breeds, no dog had greater than 24 VPCs per 24 hours, and the median value was zero. In a group of 16 healthy dogs, only two dogs had greater than 24 VPCs per recording, and they were both Boxers. These reports describe a strikingly different scenario than exists in people, where studies have shown as many as 50% of clinically normal people exhibit ectopic ventricular activity as frequent as several thousand VPCs per 24 hours.

Recently, there have been more studies evaluating Holter-derived assessment of ventricular arrhythmias in dogs with cardiovascular disease. Many of these reports identify the presence of ventricular arrhythmias as having diagnostic and prognostic value in Doberman pinschers with dilated cardiomyopathy. Others have described the Holter findings of inherited ventricular arrhythmias of German shepherd dogs and Boxers. Holter monitoring has also been performed in the assessment of ventricular arrhythmias associated with non-cardiac disease (traumatic myocarditis and splenectomy).

The importance of spontaneous variability in ventricular arrhythmias for the purposes of disease diagnosis in the previous examples is not clear. However, spontaneous variation in the frequency of ventricular arrhythmias is particularly important as it applies to response to therapy. Studies that attempt to evaluate the antiarrhythmic efficacy of a drug must take these variations into account. Some antiarrhythmic trials in dogs have been performed, but in earlier studies the response to therapy was not based on results obtained by the use of Holter monitors. More recent studies evaluating the effect of antiarrhythmic therapy have been reported using
Holter monitor evaluation. In both of these later studies, criteria used for an antiarrhythmic response were consistent with the guidelines presented here, as well as those described in the human literature.

Twenty-four hour AECG remains the most reliable method available for obtaining electrocardiographic data in people and animals. However, significant spontaneous variability makes repeatable sequential analyses of cardiac electrical activity difficult. The implication of this variability appears to be most important when assessing efficacy of antiarrhythmic therapy. The effect of this variability, however, is less clear in the application of Holter monitoring for the purpose of disease diagnosis. Future studies will hopefully aid in identifying the utility of ambulatory monitoring for this purpose.

As with any study, this report has limitations. The patients included in this analysis were all Boxers, and all were believed to have the same cardiovascular disease process (arrhythmogenic cardiomyopathy). Therefore, the results of this study may only apply to this patient population. However, as previously described, the degree of variation in this study very closely resembled the values obtained in the human literature. It may therefore be reasonable to apply these guidelines to disease processes associated with ventricular arrhythmias in other breeds with other disease processes.

Another problematic finding in this study was the disparity between the Holter evaluations in some of the dogs relative to their inclusion criteria. The inclusion criteria for this particular study was that a previous Holter (not analyzed as part of the 7 day evaluation) identified at least 500 VPCs. In four of the dogs, the number of VPCs was far less than 500, and in one case reached zero VPCs, despite the inclusion Holter.
Additionally, these dogs manifest a variability as high as 90% (and 100% in the case of the dog with zero VPCs). These findings were a surprise, and are difficult to explain. However, studies performed in people have shown that in addition to acute variations in VPC frequency, spontaneous variability also occurs over time. To determine short-term and long-term variability, two AECG recordings were taken 2-14 days apart and were then repeated 6-12 months later. Statistical analysis of recordings separated by the short interval (2-14 days) revealed variations in VPC frequency of 69% for the early pair, and 73% for the pair taken later. However, when comparing two recordings separated by the long interval (6-12 months), variability in VPC frequency was as high as 98-100%. This longer term variability may provide some explanation for the difference seen between the inclusion Holter, and those obtained during the 7-day recording period. Great care must be taken, therefore, in assessing Holter recordings taken many months apart.
Footnotes

\(^a\) Cardiocorder cassette recorder, Del Mar, Irvine CA

\(^b\) Accuplus Holter analysis system, Del Mar, Irvine CA
Reference List


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


CHAPTER 2

SIGNAL-AVERAGED ELECTROCARDIOGRAPHY

Introduction

Arrhythmogenic cardiomyopathy of Boxers, which has also been referred to as Boxer Cardiomyopathy and familial ventricular arrhythmias of Boxers, is an inherited disease of Boxers that predisposes to the development of ventricular arrhythmias, resulting in syncope or sudden death.\(^1,2\) A subset of dogs with this disease may also develop myocardial failure with progression to congestive heart failure; a condition that more closely resembles dilated cardiomyopathy in dogs.\(^1\) Dogs that manifest ventricular arrhythmias may never develop clinical signs, may remain asymptomatic for a variable length of time before developing clinical signs, or may experience sudden death as the first (and only) manifestation of the disease. Diagnosis of the disease is typically achieved by the use of routine in-hospital electrocardiography, ambulatory electrocardiography (Holter monitoring), and 2-dimensional echocardiography. Standard diagnostic evaluation of these dogs enables, in most (but not all) instances, the identification affected individuals. However, the identification of affected individuals that are at risk of developing clinical signs (i.e. syncope and sudden death) is more problematic.
In humans, adjunctive diagnostics are used to not only aid in risk stratification, but identification of arrhythmia mechanism. Most clinically important ventricular arrhythmias that are associated with sudden death are caused by reentry.³ Reentrant mechanisms result from abnormalities in either ventricular conduction or repolarization.⁴ Identifying the presence of abnormal conduction is important in the evaluation of ventricular arrhythmias, and is achieved by both invasive and noninvasive means. Invasive programmed stimulation is commonly performed in people, and has certain advantages over less invasive diagnostics. In veterinary medicine, however, these invasive procedures are not commonly performed, and more emphasis is placed on noninvasive testing.

Signal-averaged electrocardiography (SAECG) is a non-invasive electrocardiographic technique that identifies the presence of late potentials in the QRS complex that are otherwise hidden in baseline noise during standard electrocardiography. These late potentials are believed to represent areas of abnormal conduction, which may contribute to reentry.⁵⁻⁷ Consequently, the presence of late potentials serves as pathophysiologic markers of a reentrant substrate. The purpose of this study was to perform signal-averaged electrocardiography in Boxer dogs to test the hypothesis that the presence of late potentials increases with disease severity. Thus, SAECG may prove to be a valuable noninvasive marker of severe disease, and serve to aid in the identification of at-risk individuals.
Materials and Methods

Inclusion Criteria

Boxer dogs were included for SAECG analysis if they met the following criteria: a 24 hour ambulatory ECG was obtained, a two-dimensional echocardiogram was performed to assess function and exclude congenital abnormalities, and a satisfactory signal-averaged electrocardiogram could be obtained. Dogs included in the study represent individuals that participated in a larger study as part of a screening program through The Ohio State University. A group of normal, non-Boxer dogs were also evaluated by SAECG to serve as a control population. These dogs were screened by auscultation and physical exam, and were excluded if any abnormalities were identified. Dogs of mixed breeds that were believed to include either Boxer or Doberman pinscher, or other breed at high risk for cardiomyopathy, were excluded.

Ambulatory electrocardiography and echocardiography were not performed in these dogs. Normal dogs were recruited on a voluntary basis, chiefly from the staff and students associated with the veterinary school at The Ohio State University. Informed consent was obtained for all dogs evaluated.

Ambulatory electrocardiography

Twenty-four hour ambulatory electrocardiograms were obtained using a seven lead, three channel electrode system, and acquired using an analog tape recorder. Leads were arranged to approximate the frontal leads I, II, and III (Figure 2.1). Recordings
were analyzed using a prospective software analysis algorithm with continuous user interaction by a trained cardiology research assistant under the guidance of a veterinary cardiologist. The presence and complexity of ventricular arrhythmias were identified. The total number of ventricular premature complexes (VPCs) were tabulated, and the complexity of the ventricular arrhythmia was graded according to the following scheme: grade 0 = no VPCs were identified; grade 1 = only single, monomorphic VPCs were present; grade 2 = single VPCs were identified, and were present either in a bigeminal or trigeminal pattern, or multiform complexes were identified; grade 3 = presence of couplets or triplets were present; grade 4 = runs of ventricular tachycardia (four beats or longer) or the presence of RonT phenomenon.

Cardiac ultrasonography

Cardiac ultrasound was performed in unsedated dogs positioned in right lateral recumbency. Aortic velocity was obtained with a subcostal transducer position to exclude the presence of congenital valvular or subvalvular aortic stenosis. Two-dimensional and M-mode imaging in the parasternal right and long axis views were obtained to assess cardiac dimensions and function to identify the presence of associated myocardial failure or other significant cardiac lesions. Color flow Doppler was also used when indicated to confirm or exclude the cause of any auscultable murmurs. Dogs were excluded from the study if they had aortic velocities greater than 2.25 m/s (pressure gradient greater than 20mmHg), suggestive of aortic stenosis. Dogs were also excluded if there was evidence for other congenital heart disease (i.e. pulmonic stenosis). Other exclusion criteria included additional congenital lesions or
significant acquired valvular disease; these conditions, however, were not encountered in the dogs evaluated for the study, and therefore no dogs were excluded on these bases.

**Signal Averaged Electrocardiography**

Signal averaged electrocardiography (SAECG) was similarly performed in unsedated dogs in right lateral recumbency. Electrodes were placed on the body surface after hair was clipped and skin debrided to facilitate good skin-electrode interaction. Leads were placed in such a way as to approximate Leads I, aVF, and V10, creating an orthogonal lead system comprising the X, Y, and Z axes, respectively. A commercial SAECG system was used to achieve a low noise baseline to facilitate the identification of late potentials. Data points were sampled at 2000 samples per second and were filtered with a high pass frequency cutoff of both 25 and 40 Hz. At the beginning of the recording, a template QRS beat was identified to define the "typical" morphology against which other QRS complexes would be compared to exclude both artifact and ectopic/abnormal QRS morphologies. This "template beat" was also used to identify the limits for the region of interest for baseline noise calculation; the isoelectric portion of the S-T segment was used for this purpose. QRS morphologies that exhibited a 95% homology with the predetermined template beat were accepted and included in the analysis. Successive QRS complexes were included in the averaging process until the averaged value of the baseline noise was at or below 0.75 μV. QRS complexes of each lead were signal-averaged, and then filtered using a 25 Hz and 40 Hz high pass filter. These averaged and filtered QRS complexes were summated to yield a composite filtered QRS complex. A satisfactory SAECG allowed evaluation of a sufficient number
of normal ventricular complexes so as to achieve a final noise of 0.75µV. Dogs with bundle branch block causing QRS complex widths of over 80 msec were excluded from evaluation, as identification of late potentials is complicated by pre-existing conduction defects and wide QRS complexes.\textsuperscript{9,10}

Several parameters were generated during SAECG analysis that were used to document the presence of late potentials (Figure 2.2). The width of the filtered QRS complexes (QRS\textsubscript{d}), as defined by computer determined QRS onset and offset points, increases in proportion to the severity of late potentials. The duration of time that the voltage of the terminal QRS complex remains below 40µV before its termination is termed the low amplitude signal (LAS). As with the QRS\textsubscript{d}, the duration of LAS is proportional to degree of late potential. The average value of the voltage of the terminal QRS, also known as root mean square (RMS) is another estimate of late potential activity. Unlike QRS\textsubscript{d} and LAS, RMS values are inversely proportional to the degree of late potentials. QRS complexes that do not exhibit late potential activity will abruptly terminate, yielding a relatively large RMS value. Conversely, the presence of late potentials allows for a more gradual QRS offset, and results in a smaller RMS value. In this study, root mean square values were obtained over the terminal 30msec (RMS\textsubscript{30}) and 40 msec (RMS\textsubscript{40}). All parameters were calculated at high pass frequency cut-off values of both 40Hz and 25Hz. QRS\textsubscript{d} values remained constant independent of the high pass frequency filter used. Therefore, seven parameters were used: QRS\textsubscript{d}, LAS-40, RMS\textsubscript{40-40}, RMS\textsubscript{30-40}, LAS-25, RMS\textsubscript{40-25}, and RMS\textsubscript{30-25}. 

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Subject Categorization

Dogs were grouped on the basis of disease severity, as measured both by VPC frequency and by clinical assessment. Based on VPC frequency, dogs were grouped into four categories as follows: group 1 = <10 VPCs/24 hours; group 2 = 10-100 VPCs/24 hours; group 3 = 100-1000 VPCs/24 hours; group 4 = >1000 VPCs/24 hours. Based on clinical assessment, dogs were classified as either asymptomatic (A), symptomatic but not suffering a cardiac death (NCD), or those dogs dying from a cardiac cause (CD). Dogs were considered symptomatic if a history consistent with syncope was identified or if a syncopal episode was witnessed in the clinic. Dogs in the CD category were considered to have suffered a cardiac death if their death was a complication of congestive heart failure, or a sudden, unexplained death with no other known concomitant disease capable of causing such a death.

Statistical Analysis

Data were analyzed on the basis of both VPC frequency and clinical assessment. The number of VPCs were grouped as previously described (groups 1-4), and One-way Analysis of Variance was used to identify differences in each SAECG parameter based on VPC frequency group. Similarly, One-way Analysis of Variance was used to identify differences in each SAECG parameter based on the clinical status of each dog as categorized previously (A, NCD, CD). One-way Analysis of Variance was also used to identify differences between the groups of dogs based on clinical status and VPC frequency (absolute numbers, not grouped). Student’s t-test was used to identify differences in SAECG parameters between dogs that were symptomatic (CD and NCD)
and those that were asymptomatic. A Student’s t-test was also used to identify differences in SAECG parameters between symptomatic dogs that died from their disease (CD) and symptomatic dogs that did not (NCD). Non-parametric tests were used when indicated (if the data did not exhibit normal distributions). Significance level was tested at $\alpha = 0.05$. Tukey’s post hoc tests were performed when a significant difference was identified.

Results

A total of 89 Boxer dogs satisfied the inclusion criteria, and one additional dog was included for a total of 90 dogs. The additional dog that was included died before a 24 hour ambulatory ECG could be recorded, but a signal-averaged ECG and echocardiogram were obtained prior to death. He was included because the information obtained from echocardiography and in hospital ECG were sufficient to accurately diagnose and classify his disease, and a Holter would not have provided additional information in this particular dog. In addition, a total of 49 normal, non-Boxer dogs were evaluated as a control group.

Of the 90 Boxer dogs, 33 were male (37%) and 57 were female (63%). The age of the dogs ranged from 1 to 12 years, with a median of 5 years. The number of VPCs ranged from 0 to 23,699 VPCs/24 hours, with a median of 7 per 24 hour period. Based on VPC frequency, 48 dogs (53%) were classified into group 1 (<10 VPCs/24 hours), 17 dogs (19%) were placed into group 2 (10-100 VPCs/24 hours), 8 dogs (9%) were placed into group 3 (100-1000 VPCs/24 hours), and 17 dogs (19%) were placed into group 4 (>1000 VPCs/24 hours). When grouped by clinical status, 69 dogs (77%) were
asymptomatic (A), 12 dogs (13%) were symptomatic but did not die of cardiac causes (NCD), and 9 dogs (10%) suffered a cardiac death (CD), either syncope or CHF.

Descriptive data for both Boxer and normal, non-Boxer dogs for each SAECG parameter is presented in Tables 2.1 and 2.2, respectively. In addition to minimum, maximum, mean and median values, the 90th and 95th percentile values are presented for parameters that increase with degree of late potential severity (QRSd, LAS). For parameters that vary inversely with the degree of late potentials (RMS40 and RMS30), the 5th and 10th percentile are presented.

The 90th/10th and 95th/5th percentiles for each SAECG parameter from the normal dogs were used to calculate sensitivity, specificity, and positive and negative predictive values for the Boxer dogs. These results are presented in Tables 2.3 and 2.4. Finally, the values that optimize sensitivity/specificity and predictive values are presented in Tables 2.5 and 2.6.

Results of the One-way Analysis of Variance for SAECG parameters, based on both VPC frequency and clinical status groups, are summarized in Table 2.7. Relative to VPC frequency, significant differences were identified for RMS40 at both 40 and 25Hz and RMS30 at 40Hz, primarily due to differences between groups 1 and 4. For clinical status, significant differences were identified for all seven SAECG parameters. Post hoc evaluation revealed that the CD group was different than the A group in all parameters, and that the A and NCD group were not different in any parameter. Differences were identified between the CD and NCD group in all parameters except LAS at 40 and 25Hz. When clinical status was considered as either symptomatic (CD or NCD) or asymptomatic, differences were identified in all SAECG parameters except

52
QRSd and LAS at 40Hz. When comparing differences in VPC frequency between dogs grouped by clinical status, the nonparametric ANOVA on ranks was required due to the non-normal distribution of VPC frequency. A significant difference was identified between the groups, and post hoc evaluation (Dunn’s method) identified the major difference was between group A and CD. Examples of SAECGs taken from a normal dog and one taken from a dog that subsequently died are presented in Figure 2.3.
Figure 2.1. Position of electrodes for Holter placement as seen from both the left and right sides. There are 7 electrodes; 3 pair to create 3 bipolar leads (or channels), and 1 to serve as ground. Channel 1 consists of the red electrode as positive (+) and the white electrode as negative (-), approximating lead II. Channel 2 consists of the brown electrode as positive (+) and the black electrode as negative (-), approximating lead III. Channel 3 consists of the orange electrode as positive (+) and the blue electrode as negative (-), approximating lead I. The green electrode serves as ground.
Figure 2.2. Schematic of a filtered QRS (fQRS) with a description of the basic SAECG parameters: duration of the filtered QRS (QRSd), the duration of signal below 40uV (LAS40), and voltage of terminal 40 and 30 msec (RMS40 and RMS30, respectively). Figure taken from Steinberg JS and Bigger JT, Jr. Importance of the endpoint of noise reduction in analysis of the signal-averaged electrocardiogram. *Am J Cardiol* 1989;63:556-60.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Median</th>
<th>90/10%</th>
<th>95/5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRSd (msec)</td>
<td>49.5</td>
<td>81.5</td>
<td>66.7</td>
<td>67.2</td>
<td>74</td>
<td>75.5</td>
</tr>
<tr>
<td>LAS40 (msec)</td>
<td>0</td>
<td>27</td>
<td>15</td>
<td>15.2</td>
<td>22.8</td>
<td>23.5</td>
</tr>
<tr>
<td>RMS40-40 (uV)</td>
<td>41.1</td>
<td>435.5</td>
<td>238.7</td>
<td>238</td>
<td>120.7</td>
<td>78.5</td>
</tr>
<tr>
<td>RMS30-40 (uV)</td>
<td>22.1</td>
<td>404.8</td>
<td>171</td>
<td>159</td>
<td>71.3</td>
<td>44.5</td>
</tr>
<tr>
<td>LAS25 (msec)</td>
<td>0</td>
<td>22</td>
<td>9.9</td>
<td>10.2</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>RMS40-25 (uV)</td>
<td>63.9</td>
<td>710.9</td>
<td>375.1</td>
<td>354.8</td>
<td>187.6</td>
<td>143.6</td>
</tr>
<tr>
<td>RMS30-25 (uV)</td>
<td>30.5</td>
<td>601.5</td>
<td>259.2</td>
<td>246.7</td>
<td>98.6</td>
<td>54.7</td>
</tr>
</tbody>
</table>

Table 2.1. Descriptive data for SAECG parameters in 90 Boxers. For parameters that increase in value with degree of late potentials, the 90th and 95th percentiles are reported. For parameters that decrease in value with degree of late potentials, the 10th and 5th percentiles are reported.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Median</th>
<th>90/10%</th>
<th>95/5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRSd (msec)</td>
<td>53</td>
<td>83</td>
<td>63.9</td>
<td>63</td>
<td>76.5</td>
<td>78.5</td>
</tr>
<tr>
<td>LAS40 (msec)</td>
<td>4.5</td>
<td>25</td>
<td>14</td>
<td>14</td>
<td>20.1</td>
<td>24.5</td>
</tr>
<tr>
<td>RMS40-40 (uV)</td>
<td>169.1</td>
<td>625</td>
<td>342.6</td>
<td>337.5</td>
<td>207</td>
<td>175.1</td>
</tr>
<tr>
<td>RMS30-40 (uV)</td>
<td>35.5</td>
<td>459.4</td>
<td>229.8</td>
<td>220.1</td>
<td>75</td>
<td>62.1</td>
</tr>
<tr>
<td>LAS25 (msec)</td>
<td>29</td>
<td>22</td>
<td>7.2</td>
<td>7</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>RMS40-25 (uV)</td>
<td>271.2</td>
<td>919.1</td>
<td>534.4</td>
<td>530.4</td>
<td>321.2</td>
<td>280.2</td>
</tr>
</tbody>
</table>

Table 2.2. Descriptive data for SAECG parameters in 49 normal, non-Boxer dogs. For parameters that increase in value with degree of late potentials, the 90th and 95th percentiles are reported. For parameters that decrease in value with degree of late potentials, the 10th and 5th percentiles are reported. These values are used subsequently for calculations of sensitivity, specificity, and predictive value for the Boxer dogs.
Parameter & 90/10% & Sensitivity & Specificity & PPV & NPV \\
--- & --- & --- & --- & --- & --- \\
QRSd (msec) & $>76.5$ & 44\% & 100\% & 100\% & 94\% \\
LAS40 (msec) & $>20.1$ & 66.70\% & 87.60\% & 37.50\% & 96\% \\
RMS40-40 (uV) & $<207$ & 100\% & 65\% & 24.30\% & 100\% \\
RMS30-40 (uV) & $<75$ & 66.60\% & 96.30\% & 66.60\% & 96.30\% \\
LAS25 (msec) & $>14$ & 55.60\% & 81.50\% & 25\% & 94.30\% \\
RMS40-25 (uV) & $<321.2$ & 100\% & 65.40\% & 24.30\% & 100\% \\
RMS30-25 (uV) & $<119.3$ & 66.70\% & 91.40\% & 46.20\% & 96.10\% \\

Table 2.3. Sensitivity, specificity, positive and negative predictive values are presented for cut-off values at the 90th or 10th percentiles of SAECG parameters (for normal dogs). For parameters that increase in value with degree of late potentials, the 90th percentile is used; for parameters that decrease in value with degree of late potentials, the 10th percentile is reported.

Parameter & 95/5% & Sensitivity & Specificity & PPV & NPV \\
--- & --- & --- & --- & --- & --- \\
QRSd (msec) & $>78.5$ & 11\% & 100\% & 100\% & 91\% \\
LAS40 (msec) & $>24.5$ & 33\% & 100\% & 100\% & 93\% \\
RMS40-40 (uV) & $<175.1$ & 100\% & 79\% & 35\% & 100\% \\
RMS30-40 (uV) & $<62.1$ & 55.60\% & 97.50\% & 71.40\% & 95.20\% \\
LAS25 (msec) & $>15$ & 55.60\% & 87.60\% & 33.30\% & 94.70\% \\
RMS40-25 (uV) & $<280.2$ & 100\% & 80\% & 36\% & 100\% \\
RMS30-25 (uV) & $<107.5$ & 66.70\% & 93.80\% & 54.50\% & 96.20\% \\

Table 2.4. Sensitivity, specificity, positive and negative predictive values are presented for cut-off values at the 95th or 5th percentiles of SAECG parameters (for normal dogs). For parameters that increase in value with degree of late potentials, the 95th percentile is used; for parameters that decrease in value with degree of late potentials, the 5th percentile is reported.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>level to minimize false positives (maximize specificity)</th>
<th>Number of CD dogs captured (n=9)</th>
<th>Number of false positives</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>Specificity</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRSd (msec)</td>
<td>≥77</td>
<td>4</td>
<td>0</td>
<td>44%</td>
<td>100%</td>
<td>100%</td>
<td>94%</td>
</tr>
<tr>
<td>LAS40 (msec)</td>
<td>≥23</td>
<td>6</td>
<td>3</td>
<td>67%</td>
<td>67%</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>RMS40-40 (uV)</td>
<td>≤84</td>
<td>5</td>
<td>1</td>
<td>56%</td>
<td>83%</td>
<td>99%</td>
<td>95%</td>
</tr>
<tr>
<td>RMS30-40 (uV)</td>
<td>≤67</td>
<td>6</td>
<td>2</td>
<td>67%</td>
<td>75%</td>
<td>98%</td>
<td>96%</td>
</tr>
<tr>
<td>LAS25 (msec)</td>
<td>&gt;18</td>
<td>3</td>
<td>4</td>
<td>33%</td>
<td>43%</td>
<td>95%</td>
<td>93%</td>
</tr>
<tr>
<td>RMS40-25 (uV)</td>
<td>≤147</td>
<td>5</td>
<td>1</td>
<td>56%</td>
<td>83%</td>
<td>99%</td>
<td>95%</td>
</tr>
<tr>
<td>RMS30-25 (uV)</td>
<td>≤76</td>
<td>5</td>
<td>2</td>
<td>56%</td>
<td>71%</td>
<td>98%</td>
<td>95%</td>
</tr>
</tbody>
</table>

Table 2.5. Values for each SAECG parameter that optimize specificity and minimize number of false positives are presented. The number of CD dogs correctly identified (out of 9 total dogs), the number of false positives, sensitivity, positive predictive value (PPV), specificity, and negative predictive value (NPV) for each value are shown.
Table 2.6. The value for each SAECG parameter that correctly identifies all 9 CD dogs, maximizing sensitivity and negative predictive value (NPV) is presented. The number of false positives, specificity, and positive predictive value (PPV) for each value are shown.
<table>
<thead>
<tr>
<th>VPC freq. (Groups 1-4)</th>
<th>SAECG Parameter</th>
<th>p-value</th>
<th>post hoc test</th>
<th>Groups different</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QRSd</td>
<td>NS</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>LAS40</td>
<td>NS</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>RMS40-40</td>
<td>&lt;0.001</td>
<td>Tukey</td>
<td>1 and 4</td>
</tr>
<tr>
<td></td>
<td>RMS30-40</td>
<td>0.018</td>
<td>Tukey</td>
<td>1 and 4</td>
</tr>
<tr>
<td></td>
<td>LAS25</td>
<td>NS</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>RMS40-25</td>
<td>&lt;0.001</td>
<td>Tukey</td>
<td>1 and 4</td>
</tr>
<tr>
<td></td>
<td>RMS30-25</td>
<td>NS</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Status (A, NCD, CD)</td>
<td>QRSd</td>
<td>0.003</td>
<td>Tukey</td>
<td>CD/A, CD/NCD</td>
</tr>
<tr>
<td></td>
<td>LAS40</td>
<td>0.003</td>
<td>Tukey</td>
<td>CD/A only</td>
</tr>
<tr>
<td></td>
<td>RMS40-40</td>
<td>&lt;.001</td>
<td>Tukey</td>
<td>CD/A, CD/NCD</td>
</tr>
<tr>
<td></td>
<td>RMS30-40</td>
<td>&lt;.001</td>
<td>Tukey</td>
<td>CD/A, CD/NCD</td>
</tr>
<tr>
<td></td>
<td>LAS25</td>
<td>0.015</td>
<td>Tukey</td>
<td>CD/A only</td>
</tr>
<tr>
<td></td>
<td>RMS40-25</td>
<td>&lt;.001</td>
<td>Tukey</td>
<td>CD/A, CD/NCD</td>
</tr>
<tr>
<td></td>
<td>RMS30-25</td>
<td>0.001</td>
<td>Tukey</td>
<td>CD/A, CD/NCD</td>
</tr>
</tbody>
</table>

Table 2.7. Summary of statistical tests to identify differences in SAECG parameters for dogs grouped by VPC frequency or clinical status. See text for category definitions.
Figure 2.3. An example of a SAECG from a normal dog (A) compared to an abnormal SAECG from an affected Boxer (B) with late potentials that subsequently died. The Boxer with the abnormal SAECG and late potentials had an increased QRSd and LAS, with a decreased RMS40.
Discussion

To our knowledge, this is the first study to evaluate the utility of SAECG in the assessment of arrhythmogenic cardiomyopathy in Boxer dogs. The results of this study document that signal-averaged electrocardiography is a useful test in the evaluation of this disease in Boxers. We were able to identify differences between groups of dogs categorized by disease severity, measured both by quantification of VPC frequency and clinical status. In most instances, the majority of the difference was identified in the group that was most severely affected. SAECG proved useful in identifying those dogs that are most likely to suffer a cardiac death, as well as identifying differences between dogs that were asymptomatic and those that were symptomatic (manifesting either as syncope or cardiac death). For each parameter, values could be identified that were associated with either high sensitivity (with high negative predictive value) or high specificity (with high positive predictive value) for a cardiac death; however, numerous false positives and false negatives occurred.

Signal-averaged ECG has been used infrequently in veterinary medicine in the evaluation of canine heart disease. In the assessment of dilated cardiomyopathy in Doberman pinschers\textsuperscript{11}, and in 4 dogs with sustained ventricular arrhythmias\textsuperscript{12}, the presence of late potentials exhibited the ability to potentially identify dogs which were more likely to suffer a cardiac death. In these dogs, the manifestation of cardiac disease was similar to the subset of Boxers that either have severe arrhythmias or develop systolic dysfunction and congestive heart failure.

In human medicine, the evaluation and assessment of patients with ventricular arrhythmias employs a variety of diagnostic approaches. A major goal in the evaluation
of such patients is to identify those individuals who are at the highest risk of suffering adverse events, particularly sudden death. Diagnostic testing used for these purposes include Holter evaluation, exercise testing, radionucleotide ventrigulography, and programmed stimulation. With the exception of Holter monitoring, the usefulness of these other modalities is limited in veterinary medicine due to a combination of expense, invasiveness, and patient compliance. Consequently, this has resulted in the search for less invasive testing that may be more readily performed in veterinary patients.

Signal-averaged electrocardiography is a high resolution ECG that allows the identification of late potentials occurring in the terminal portion of the QRS complex. The presence of late potentials is believed to represent areas of abnormal conduction that contributes to anatomic reentry. Reentrant circuits are considered the principal causes of the arrhythmias that predispose to sudden death. Thus, the presence of late potentials is a marker for an arrhythmic substrate, and may identify individuals at risk for adverse arrhythmic events. Identification of late potentials has been documented as a predictor of adverse arrhythmic events, particularly in conjunction with Holter monitoring and assessment of ventricular function. Diseases for which SAECG has been used in the evaluation of ventricular arrhythmias include dilated cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic right ventricular dysplasia cardiomyopathy, ischemic heart disease, and nonspecific causes of syncope.

The utility of signal-averaged electrocardiography in human medicine is not limited as a single diagnostic test, but is frequently used for screening of patients to
identify at-risk individuals in whom additional testing is indicated, particularly the electrophysiologic study.\textsuperscript{6,7,10} As stated previously, the expense and invasive nature of EP testing is, for the most part, prohibitive in veterinary medicine. In human studies, the presence of late potentials as identified by SAECG has predicted the development of inducible ventricular arrhythmias on EP testing, and SAECG may be used as an independent diagnostic test.\textsuperscript{6,7,9}

The identification of late potentials on SAECG is dependent on the technique used, as normal values are highly dependent on recording and filtering technique.\textsuperscript{6} Identification of late potentials can be performed several ways, including temporal (time), spectral (frequency), and spatial averaging techniques.\textsuperscript{6,7} Spatial averaging allows identification of late potentials of a single QRS complex by placing multiple electrodes over the body surface. Signal noise is reduced by the comparing closely spaced electrodes, with the assumption that cardiac electrical activity between these electrodes is more consistent than random noise. In this way, random noise can be identified as differences between closely spaced electrodes, allowing the more consistent cardiac generated potentials to persist. The advantage of such a system is that it allows identification of late potentials for a single beat, which may be particularly important in the presence of significant arrhythmia. It is also particularly useful when late potential activity fluctuates as a result of periodic conduction abnormalities. The disadvantage is the difficulty of the technique, which requires that the patient be electrically shielded. Additionally, electromyographical noise, which tends to be more consistent and coherently related to cardiac electrical activity, may not be attenuated to the same degree as more random electrode/amplifier noise.\textsuperscript{7}
Spectral, or frequency domain, analysis is achieved by analyzing the frequency content of the terminal QRS signal. The generation of late potentials, caused by abnormal fractionated conduction through diseased myocardium, not only causes changes in conduction velocity, but also contains higher frequency components. The evaluation of the frequency content of the region of interest (i.e. the terminal QRS) is achieved by utilizing fast Fourier transform (FFT) to decompose a signal into its frequency components. The identification of high frequency content within a signal may correlate with patients that have ventricular arrhythmias; however, this technique has been shown to lack reproducibility and is very sensitive to recording parameters. Furthermore, frequency domain analysis has not been shown to be more informative compared to temporal averaging. A more useful application of spectral analysis is in conjunction with temporal analysis, or so-called spectrottemporal averaging. For these reasons, frequency analysis was not performed in this study.

In this study, temporal, or time domain, analysis was used for identification of late potentials. Unlike spectral analysis, this technique identifies late potentials by reducing baseline noise through averaging of successive QRS complexes and application of digital filters. The averaging process allows repeatable, consistent signals to persist, while eliminating random signals. Temporal averaging is based on the principle that the late potentials are fixed in time relative to overall cardiac depolarization, and that noise is a random event. With these assumptions in place, baseline noise can be reduced as a function of the number of beats averaged, proportional to \( \sqrt{n} \), where \( n \) = number of averaged beats. Other factors involved include the initial noise level, the degree of muscular activity, and adequacy of skin.
An orthogonal lead system is used to record the ECG, and each lead is individually averaged until a specified end point. Once the endpoint is reached, the individual leads are mathematically combined into a vector, according to the formula \( \sqrt{x^2 + y^2 + z^2} \), where \( x = \text{lead I} \), \( y = \text{lead aVF} \), and \( z = \text{lead V}_{10} \). Digital filters are applied to this composite QRS to facilitate the identification of late potentials, creating a filtered QRS complex. High pass filters, which allow only certain frequencies above a particular range to pass unimpeded, are used to eliminate low frequency components, such as baseline drift, and better identify QRS onset and offset. Low pass filters, which allow only certain frequencies below a particular range to pass unimpeded, are used to eliminate high frequency noise, such as electromyographic artifact.

An important feature of time domain analysis is maintaining a consistent temporal relationship between the QRS inscription and high frequency activity such as noise or late potentials. This is achieved with a marker referred to as the fiducial point, which serves as a reference point for subsequent noise/late potentials. Technical limitations of temporal averaging are largely dependent on this relationship. Signals that are not fixed relative the fiducial point, or movement of the fiducial itself, called jitter, can distort and alter the presence of late potentials. Because multiple QRS complexes are included in the averaging process, the individuals beats must be almost identical. Therefore, premature complexes, aberrant conduction (such as bundle branch block), and noisy beats need to be excluded from the analysis.

Another important factor influencing the time domain analysis is the manner of end-point determination. The end-point of the procedure can be defined by either a pre-determined number of averaged beats, or by a pre-determined noise level.
Both methods have been used, but end-point determination based on noise level may allow for better reproducibility.\(^7,16\) The ideal level of end-point noise has not been absolutely defined, but is likely to be between $1\mu\text{V}$ and $0.3\mu\text{V}$.\(^5,7,10,16-18\) There exists a "trade-off" between accuracy associated with lower levels of noise, and reproducibility associated with higher levels of noise.\(^6,7,18\) For these reasons, and to increase the chance that a particular noise level could consistently be achieved in unsedated dogs, we chose to determine an end-point using a noise level of $0.75\mu\text{V}$, regardless of the number of beats necessary to achieve that level.

Boxers with arrhythmogenic cardiomyopathy manifest pathologic and clinical similarities to human patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC). ARVC in people is an inherited disease in which affected individuals are at risk for the development of ventricular arrhythmias, and are also at an increased risk of sudden death. Myocardial infiltration of fibrous and adipose tissue is the predominant pathologic finding in these patients, and the degree of infiltration correlates to severity of disease.\(^5,10,19\) The presence of late potentials identified in patients with ARVC is believed to arise from conduction abnormalities secondary to myocardial fibrofatty infiltration, and the presence of late potentials is dependent on the severity of disease.\(^5\) This finding supports the conclusion that SAECG is most useful in identifying abnormal conduction and increased risk of adverse arrhythmic events in patients with the more severe forms of the disease, suggestive of more significant myocardial infiltration.\(^5,10\) This pattern was also identified in the current study of Boxer dogs, and lends credence to the similarities between the two diseases. In our current study, the dogs with more severe disease, measured by either
VPC frequency or clinical status, were more likely to have late potentials on their SAECG. Those dogs with less severe disease were often indistinguishable from unaffected, asymptomatic dogs.

Several limitations exist in the current study. As with many clinical studies in veterinary medicine, this study suffers due to the relatively low number of dogs included. This is particularly the case with symptomatic dogs. Based on VPC frequency, less than 20% of the dogs had >1000VPCs/24 hrs, and almost 50% had less than 10VPCs/24 hrs. Less than 25% were symptomatic, and of those only 10% suffered a cardiac death. Because of the low numbers, no attempt was made to distinguish between dogs that had died with systolic dysfunction in addition to an arrhythmia from those that died of without myocardial failure. As the number of dogs evaluated increases, a distinction between these groups may be possible.

The identification of a particular Boxer as either affected or unaffected can be very difficult, as no clear diagnostic criteria currently exists for this disease in Boxers. Consequently, normal values were generated from clinically normal, non-Boxer dogs. It may be more desirable to compare normal Boxers to abnormal Boxers, but the uncertainty of identifying a Boxer as “normal” or “abnormal” precludes this distinction at this time. Thus the designation of symptomatic or asymptomatic was chosen, and SAECG parameter values were derived from a clinically normal, non-Boxer control group that was screened for heart disease.

The distinction between symptomatic and asymptomatic dogs was not absolute, even in the most severely affected groups. Some dogs that died from cardiac causes had fewer late potentials than asymptomatic animals that were otherwise clinically normal.
Reasons for the presence of late potentials in an otherwise healthy individual may be due to technical artifacts or noise level. It is possible that the noise level chosen was too low, or spurious signals persist in the averaging process that do not necessarily represent a true late potential.\(^6,^10\) Alternatively, the presence of late potentials documents the existence of an anatomic substrate for arrhythmia formation. However, the presence of a reentrant circuit requires additional modifiers or triggering mechanisms to produce reentry and ventricular arrhythmias.\(^10\) Up to 4-6\% of normal controls in people manifest the presence of late potentials.\(^5^,^7\) The absence of late potentials in otherwise affected individuals may simply reflect a decreased amplitude in potential, or abnormal potentials that are "buried" in a baseline with too high a noise level.\(^5\) It is also possible that the mechanism for arrhythmia formation differs between subsets of patients, and that in some patients the arrhythmias are automatic in nature, and not reentrant.\(^5\)

Despite these limitations, the conclusions of this study support the findings observed in people with ARVC that late potentials do occur in patients with arrhythmic disease, and the presence of abnormal electrical activity parallels disease severity. We also conclude that SAECG has clinical utility in Boxers with arrhythmogenic cardiomyopathy, and future studies may be necessary to determine if different subsets of these dogs can be differentiated based on the presence of late potentials.
Footnotes

a Cardiocorder cassette recorder, Del Mar, Irvine CA

b Accuplus Holter analysis system, Del Mar, Irvine CA

c System 5, VingMed, Denmark

d Pagewriter Xli with SAECG module M1754, Hewlett Packard,
Reference List


CHAPTER 3

QT DISPERSION

Introduction

Arrhythmogenic cardiomyopathy of Boxer dogs is commonly encountered in veterinary medicine, and is characterized by the development of ventricular tachyarrhythmias resulting in sudden death.¹ ² The mechanism of the arrhythmia in Boxer dogs is unknown. There are three mechanisms postulated for the genesis of ventricular tachyarrhythmias, including enhanced automaticity, triggered activity, and reentry. In people, the predominant mechanism of ventricular tachyarrhythmias resulting in sudden cardiac death is believed to be reentry.³ Reentrant circuits are created when adjacent myocardial tissues exhibit different electrophysiologic properties, allowing for the development of abnormal impulse conduction. The disparity in electrophysiologic properties may be due to structural abnormalities or functional changes. Anatomic abnormalities, including hypertrophy, fibrosis and ischemia, create areas of differing excitability and electrophysiologic properties resulting from the presence of abnormal tissue. Functional reentry occurs in the absence of any identifiable anatomic substrate, presumably as the result of inherent electrophysiologic differences in membrane and ionic properties of excitable tissue.
Differences in the electrophysiologic properties of excitable tissue may manifest as inhomogeneity (or heterogeneity) of ventricular depolarization and repolarization. The QT interval, measured from the surface electrocardiogram, is an electrocardiographic parameter of ventricular repolarization. The duration of the QT interval is not identical in all leads; this interlead difference between the maximum and minimum QT durations is known as QT dispersion. It is hypothesized that the degree of variation in QT interval duration among leads (dispersion) reflects regional differences of ventricular repolarization. Increased QT dispersion reflects increased inhomogeneity of ventricular repolarization. Dispersion of ventricular repolarization reduces the threshold for ventricular fibrillation and facilitates the development of reentrant ventricular tachyarrhythmias. Consequently, QT dispersion may be used as a "marker of arrhythmogenicity", and may support a reentrant mechanism.

QT dispersion may be an indicator of a reentry and serve as a non-invasive means to identify electrical dispersion in Boxer dogs with FVA. This could provide evidence for the presence of an arrhythmogenic substrate, and be useful in identifying dogs at risk for arrhythmia development and sudden cardiac death. In humans, QT dispersion has been used with some success for identifying patients at risk for developing ventricular tachyarrhythmias associated with a number of cardiovascular diseases, including chronic heart failure (CHF), hypertrophic cardiomyopathy, myocardial ischemia, aortic stenosis, mitral valve prolapse, and long QT syndrome. The development of a non-invasive diagnostic test capable of identifying patients who are most severely affected with FVA that may be at highest risk for developing
ven tricular tachy arrhythmias and/or sudden death would represent a significant advancement in the management of Familial ventricular arrhythmias in Boxer dogs.

The purpose of this study was to (1) measure QT interval durations and QT dispersion in a group of Boxer dogs, and (2) determine if QT parameters correlate with indices of disease severity of Familial Ventricular Arrhythmias, including the number of ventricular premature complexes, grade of arrhythmia, and left ventricular function (as measured by percent fractional shortening).

Materials and Methods

Animal Selection

Twenty-five mature Boxer dogs were recruited for evaluation and evaluated based upon physical examination, 12-lead electrocardiograms (ECGs), ambulatory electrocardiography and 2-dimensional echocardiography. Historical information regarding exercise tolerance and episodes of syncope was also obtained.

12-lead electrocardiography

A 12-lead ECG was obtained in unsedated dogs positioned in right lateral recumbency. Tracings were recorded at a paper speed of 25 and 50 mm/sec, with a gain of 10 mm/mV. In some instances, the recording of precordial leads overlapped with the limb leads, and in these cases the gain of the precordial leads was reduced to 5 mm/mV. QT intervals were measured manually as the duration from the onset of the QRS complex to the termination of the T wave. Three consecutive beats were measured, and the RR interval immediately preceding each complex was recorded. A heart rate-
corrected QT interval (QTc) was calculated for each beat as the duration of the QT interval divided by the cube root of the RR interval (measured in seconds) of the preceding cardiac cycle. A mean QT and QTc was calculated for each lead based on the three measured beats, from which QT and QTc dispersion were determined. QT and QTc dispersion were defined as the difference between the maximum and minimum value of the mean QT and QTc for each lead, respectively. In addition to QT and QTc dispersion, an average value for QT and QTc (QTavg and QTcavg) values were calculated for each dog using the mean values from each of the 12 leads.

24-hour ambulatory electrocardiography

Twenty-four hour ambulatory electrocardiography was performed using three-channel cassette recorders and analyzed with a Holter analysis system with prospective, user interaction. Total number of ventricular premature complexes (VPCs) and duration of recording were obtained. Only those recordings at least 20 hours in duration were included. If VPCs were identified, the arrhythmia was graded based on a modification of the Lown grading scheme as follows: grade 1 = single, uniform VPCs only; grade 2 = presence of bigeminy, trigeminy, or multiform VPCs; grade 3 = presence of couplets or triplets, and grade 4 = presence of RonT phenomenon or ventricular tachycardia. Animals in which no VPCs were identified were classified as grade zero.

Echocardiography

Two-dimensional echocardiography was performed in unsedated dogs positioned in right lateral recumbency, using standard techniques. Percent fractional
shortening (FS%) was calculated as the percent difference between diastolic (LVIDd) and systolic (LVIDs) left ventricular dimensions according to the formula $FS\% = \frac{(LVIDd - LVIDs)}{LVIDd} \times 100$.\textsuperscript{17,18}

**Statistical evaluation**

QT parameters (QT and QTc dispersion, and average QT and QTc) were evaluated for correlation to total number of VPCs, arrhythmia grade, and percent fractional shortening. For the purpose of statistical calculations, arrhythmia grade was converted to a numeric ordinal scale as described. The non-parametric Spearman correlation coefficient was used to identify significant correlation between the QT parameters and indices of disease severity. A Mann-Whitney rank sum test was used to identify a difference between QT dispersion and QTc dispersion. In all cases, statistical significance was tested at $\alpha=0.05$.

**Results**

Twelve-lead electrocardiography, ambulatory electrocardiography, and echocardiography were performed in 25 Boxer dogs (20 females, 5 males). Descriptive statistics for total number of VPCs on 24-hour ambulatory ECG (VPC#), grade of arrhythmia, and percent fractional shortening (FS%), in addition to the QT parameters (QT and QTc dispersion, average QT and QTc) are shown in Table 3.1. In 22 of 25 dogs, QT dispersion (and consequently QTc dispersion) was zero. Data for the three dogs with QT dispersion not equal to zero are presented in Table 3.2. Values for QT avg and QTc avg are within one standard deviation of the mean, and indicators of disease...
severity do not suggest presence of disease. Two of the 25 dogs had a history of syncope. In both dogs, QT and QTc dispersion are zero, and QT parameters are either less than the mean, or are within one standard deviation of the mean. None of the dogs with non-zero QT dispersion had a history of syncope.

QT dispersion, QTc dispersion, average QT, and average QTc were evaluated for any correlation to VPC#, arrhythmia grade, and percent fractional shortening (FS%). No significant correlations between any of the QT parameters and the indices of disease severity were found.

Evaluation of heart rate correction for QT interval durations revealed that individual measurements of QT intervals often did not change on a beat-to-beat basis, despite beat-to-beat variation in heart rate. A Mann-Whitney rank sum test failed to identify a difference between QT dispersion and QTc dispersion.

Two of the dogs (a 6 year old male and a 10 year old female) were syncopal, and their QT, Holter, and echocardiographic parameters were as follows: both had QT dispersion of zero, and QT average (213, 200) and QTc average (230, 259) were below and within 2 standard deviations of the mean. The VPC# and grade from Holter recordings were 68 VPCs, grade 2 and 505 VPCs, grade 3, respectively. Percent fractional shortening (32, 25) was normal for each dog.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT disp(ms)</td>
<td>0</td>
<td>3.9</td>
<td>12.5</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>QTc disp(ms)</td>
<td>0</td>
<td>4.8</td>
<td>15.6</td>
<td>0</td>
<td>61.3</td>
<td>61.3</td>
</tr>
<tr>
<td>QT avg(ms)</td>
<td>213</td>
<td>218.0</td>
<td>18.6</td>
<td>180</td>
<td>253</td>
<td>73</td>
</tr>
<tr>
<td>QTc avg(ms)</td>
<td>261</td>
<td>264.5</td>
<td>21.5</td>
<td>230</td>
<td>317</td>
<td>87</td>
</tr>
<tr>
<td>Age(yr)</td>
<td>4</td>
<td>4.5</td>
<td>3.2</td>
<td>1</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>FS%</td>
<td>31.0</td>
<td>30.5</td>
<td>4.0</td>
<td>20.0</td>
<td>36.0</td>
<td>16.0</td>
</tr>
<tr>
<td>VPC#(per day)</td>
<td>5</td>
<td>2,776</td>
<td>12,489</td>
<td>0</td>
<td>62,622</td>
<td>62,622</td>
</tr>
<tr>
<td>Arrhythmia Grade</td>
<td>2</td>
<td>2.0</td>
<td>1.4</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Heart Rate(bpm)</td>
<td>111</td>
<td>109</td>
<td>19.9</td>
<td>65</td>
<td>148</td>
<td>83</td>
</tr>
</tbody>
</table>

Table 3.1. 25 Boxers with echocardiogram and 24 hour Holter. Median, mean, standard deviation (SD), minimum (min), maximum (max), and range values for QT parameters, indices of disease severity, age, and heart rate of 25 Boxer dogs evaluated by 12-lead electrocardiography, ambulatory electrocardiography and echocardiography.

<table>
<thead>
<tr>
<th>QT disp</th>
<th>QTc disp</th>
<th>QT avg</th>
<th>QTc avg</th>
<th>Age</th>
<th>Gender</th>
<th>FS%</th>
<th>VPC#</th>
<th>Arrhythmia Grade</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>61.3</td>
<td>224</td>
<td>280</td>
<td>1.5</td>
<td>M</td>
<td>28</td>
<td>1</td>
<td>1</td>
<td>110</td>
</tr>
<tr>
<td>7</td>
<td>7.9</td>
<td>223</td>
<td>262</td>
<td>2</td>
<td>F</td>
<td>36</td>
<td>6</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>40</td>
<td>50.6</td>
<td>225</td>
<td>276</td>
<td>1.5</td>
<td>F</td>
<td>35</td>
<td>24</td>
<td>2</td>
<td>111</td>
</tr>
</tbody>
</table>

Table 3.2. 3 dogs with non-zero QT dispersion. QT parameters, signalment, and indices of disease severity for the three Boxer dogs in which QT and QTc dispersion are non-zero. Values for QT avg and QTc avg are within one standard deviation of the mean, and indicators of disease severity do not suggest presence of disease.
Discussion

This is the first study to report the role of QT dispersion in the assessment of FVA in Boxer dogs. Results obtained in this study indicate that QT interval duration or dispersion of QT do not correlate with either total number of VPCs, arrhythmia grade, or percent fractional shortening. The majority of dogs had QT dispersion equal to zero, implying that substantial dispersion of repolarization was not present in this group of dogs, and would not be expected to discriminate dogs based on disease severity. There was no difference between dispersion of QT and QTc intervals, and individual measurements of QT intervals did not change on a beat-to-beat basis, despite beat-to-beat variation in heart rate resulting from respiratory sinus arrhythmia. Thus, heart rate correction of the QT interval did not appear to be necessary for QT dispersion calculation in this group of dogs.

Human studies evaluating QT dispersion have revealed potential clinical utility in predicting arrhythmic episodes or sudden death associated with cardiovascular diseases, such as coronary artery disease, hypertrophic cardiomyopathy, chronic heart failure, aortic stenosis, mitral valve prolapse, and long-QT syndrome.\textsuperscript{5,6,10-12,14} Despite studies that claim clinical usefulness of QT dispersion in identifying patients at risk for future arrhythmic episodes or sudden death, much controversy still remains. Not all studies evaluating QT dispersion have arrived at the same conclusions.\textsuperscript{19-21} Disparity in findings could result from evaluation of QT dispersion using retrospective study designs or having protocols that were subject to selection bias. This lack of standardization of the use of QT dispersion as a diagnostic test makes comparison between studies difficult, which may account for some of the differences noted.\textsuperscript{19}
One of the difficulties in evaluating QT dispersion is with the method of QT interval measurement itself. There is little difficulty in identifying the onset of the QRS, but there can be some ambiguity in determining the termination of the T wave. Low T wave amplitude, variations in T wave morphology, and the presence of a U wave are all factors that can contribute to the inability to accurately identify termination of the T wave, resulting in difficulty of QT interval measurement. Some studies, in an effort to eliminate this uncertainty, have excluded leads in which these problems exist. Because QT dispersion is the difference between the largest and smallest QT interval between leads, eliminating one or more leads may have an effect on its value. Other efforts used to avoid complications associated with accurate T wave termination include measurement of the QT interval from the onset of the QRS complex to the peak of the T wave (QTpeak). The peak of the T wave can be more reliably identified than the end of the T wave, and is thus associated less ambiguity. Studies using different techniques to improve the accuracy of QT measurement have also created a lack of standardization that contributes to the inability to compare studies.

Results of this study indicate a lack of correlation between QT parameters and indices of disease severity. As in other studies, the methods used may contribute to these findings. In this study, QT interval durations were measured manually. Manual measurement in previous studies has been aided by the use of a digitizer coupled with magnification to improve resolution and enhance accurate identification of T wave termination. Using these criteria, error of measurement has been determined to be on the order of 20 ms. With the use of caliper measurement in this study, accuracy of measurement was estimated to be 0.25 mm, which corresponds to 10 ms for recordings.
made at 25mm/sec, and 5 ms for recordings made at 50mm/sec. However, this is an unsubstantiated estimate, and only takes into account the perceived error of measurement with the calipers, and does not reflect accurate identification of T wave termination. In this study, we did not appreciate difficulty in identification of T wave termination, and did not observe unusual T wave morphology (biphasic T waves, notching, changes in polarity) or presence of U waves. As a result, we did not use a digitizer or magnification, nor did we calculate QT_{peak} intervals. Validation of computer-free manual measurements may be necessary to fully appreciate the degree of measurement error. However, the frequency of QT dispersion values of zero and the magnitude of non-zero measurements, suggest that measurement error is unlikely to significantly affect the conclusions of this study.

Duration of the QT interval is affected by heart rate, and QT measurements are frequently corrected for heart rate (QTc). There are many ways in which QT interval duration can be corrected for heart rate; two of the most commonly used are Bazett’s formula and Fridericia’s formula.\textsuperscript{15} Correcting for heart rate in humans may be unnecessary as adjustments based on Bazett’s formula (square root) are negligible at heart rates between 50-80 beats per minute.\textsuperscript{25} In dogs, normal heart rates are higher, with rates greater than 120 beats per minute frequently occurring in dogs in this study. Because Bazett’s formula is believed to be less accurate at higher heart rates, we chose to correct the QT interval using Fridericia’s (cube root) method.\textsuperscript{26} However, the importance of heart rate correction for the purpose of QT dispersion calculation in dogs is unclear. Furthermore, due to the relatively slow response of QT duration changes as a result of changing heart rate, the practice of measuring RR intervals immediately
preceding each beat may be complicated in the presence of respiratory sinus arrhythmia, which commonly occurs in dogs. The necessity of heart rate correction for QT dispersion is also unclear. In some investigators' opinion, the most serious drawback to the use of heart rate corrected QT durations is that studies evaluating QT dispersion identify differences between patient populations based on differences in underlying heart rate, and not true differences in repolarization. Most studies examining QT dispersion have reported values in terms of QTc, which may represent a confounding factor in the interpretation of their results.

In this study, QT and heart rate corrected QT (QTc) measurements were determined, and no difference between QT and QTc dispersion was identified. Furthermore, individual measurements of QT intervals did not change on a beat-to-beat basis, despite beat-to-beat variation in heart rate resulting from respiratory sinus arrhythmia. Therefore, the use of heart rate correction of the QT interval did not appear to be necessary for QT dispersion calculation in this group of dogs.

Limitations in this study may arise from the assumption that percent fractional shortening obtained from 2-dimensional echocardiograms and the frequency and severity (grade) of the arrhythmia taken from 24-hour ambulatory electrocardiograms are useful in quantifying the severity of FVA in Boxer dogs. The association between echocardiographic and electrocardiographic parameters and FVA has not been demonstrated. The lack of correlation between QT dispersion and the results of these diagnostic tests may imply the lack of significant heterogeneity of repolarization in dogs with FVA, or it may simply reflect the inability of these diagnostic parameters to quantify disease severity. However, these parameters are commonly used in the
diagnosis of FVA, and serve to guide therapy and provide prognostic information in the clinical management of the disease.\textsuperscript{1,2} The gender distribution in this group of dogs was skewed (20 female, 5 male), which may have influenced the results. The discrepancy most likely is a result of the fact that most animals evaluated are owned by breeders, and females were more commonly presented. However, no evidence supporting a difference in QT parameters based on gender exists in dogs. Furthermore, previous evaluation of VPC\# and arrhythmia grade did not identify significant differences based on gender.\textsuperscript{b} The low numbers of dogs available for evaluation may have also contributed to our inability to demonstrate significant correlation between QT parameters and these indices of disease severity. More studies including a greater number of animals may be needed to validate these findings.

Conclusions

The results of this study suggest that QT interval duration and QT dispersion do not correlate with indices of disease severity for FVA, including percent fractional shortening, number of VPCs, and grade of arrhythmia. Furthermore, heart rate correction of the QT interval for the purposes in calculating QT dispersion may not be necessary.
Footnotes

a. Del Mar Avionics, Irvine Ca

Reference List


Chapter 4

Heart Rate Variability

Introduction

The autonomic nervous system plays a major role in the control of the cardiovascular system, particularly in the modulation of heart rate.\textsuperscript{1-4} The degree of variation in heart rate, or heart rate variability, can be used as an assessment of autonomic influence on the heart.\textsuperscript{2-5} Increased sympathetic innervation decreases the variability of RR intervals, while parasympathetic tone increases the degree of variation in RR intervals. Sympathetic and parasympathetic tone not only has an impact on the variation in heart rate, but can also affect heart rhythm. Autonomic balance influences the occurrence and severity of ventricular arrhythmias, with sympathetic tone exacerbating these arrhythmias, and parasympathetic tone having a protective effect.\textsuperscript{5}

Reduced heart rate variability, associated with increased sympathetic tone and withdrawal of parasympathetic tone, has been used to predict mortality in patients with congestive heart failure and identify risk of sudden death in patients with ventricular arrhythmias.\textsuperscript{3,6-8} In veterinary medicine, analysis of heart rate variability has been used to evaluate Doberman pinschers with dilated cardiomyopathy.\textsuperscript{9-11} Most dogs with dilated cardiomyopathy develop congestive heart failure, with a smaller subset of
patients dying suddenly from cardiac arrhythmias.\textsuperscript{12,13} By contrast, Boxers manifest a
type of primary heart disease characterized by the presence of ventricular arrhythmias
and risk of sudden death, with a smaller subset of patients developing systolic
dysfunction and congestive heart failure.\textsuperscript{14} The purpose of this study was to (1)
measure heart rate variability in Boxers with and without ventricular arrhythmias, (2)
assess the ability of this method to identify Boxers based on disease severity, and (3)
use heart rate variability to determine if persistently elevated sympathetic tone is present
in affected dogs.

Materials and Methods

Patient selection

Boxer dogs were included in this study as part of a multi-phased research study
evaluating ventricular arrhythmias in Boxer dogs. All dogs were evaluated by physical
exam, standard in-hospital electrocardiography, 2-dimensional and Doppler
echocardiography, and 24-hour ambulatory electrocardiography (Holter monitoring).
Dogs were included in the study if they were over 2 years old with no evidence of
congenital heart disease.

Subject categorization

Dogs were classified according to the number of VPCs on Holter evaluation.
Dogs with $\leq$2 VPCs/24 hours were classified as unaffected (U). Dogs with $>1000$
VPCs/24 hours were classified as affected (A). A group of dogs with congestive heart
failure (HF) were also included to serve as reference group expected to have reduced
heart rate variability and increased sympathetic tone. A group of normal, non-Boxer
dogs (N) were also included to serve as a control population for the Boxers. These dogs
were evaluated by physical examination, standard in-hospital electrocardiography, and
Holter evaluation.

*Ambulatory electrocardiography*

Twenty-four hour ambulatory electrocardiograms were obtained using a seven
lead, three channel electrode system, and acquired using an analog tape recorder. Lead
were arranged to approximate the frontal leads I, II, and III (Figure 4.1). Recordings
were analyzed using a prospective software analysis algorithm with continuous user
interaction by a trained cardiology research assistant under the guidance of a
veterinary cardiologist.

*Heart rate variability analysis*

Analysis of heart rate variability was performed on 24-hour Holter recordings
using a commercial laboratory. Heart rate variability analysis was performed in the
time domain. Parameters that were calculated included the average normal R-R interval
(mean RR) and the standard deviation of the normal R-R intervals (SDNN) over the
entire recording period. Additional parameters were calculated by dividing the 24-hour
recording into 288 separate five minute periods. The mean and standard deviation (SD)
of R-R intervals for each five minute period were then calculated. The standard
deviation of the mean R-R interval for each five minute period (SDANN) and the
average of the standard deviation of R-R intervals for each five minute period (ASDNN) were calculated for the entire 24-hour ambulatory (Holter) recording.

**Statistical evaluation**

Data were analyzed by comparing the mean value for each time domain parameter between groups of dogs using a one-way Analysis of Variance (ANOVA). Differences were evaluated between the four groups of dogs, including normal, non-Boxer dogs (N), affected (A) and unaffected (U) Boxers, and Boxers with heart failure (HF). Significance was defined at $\alpha \leq 0.05$. If a statistically significant difference was identified, a post-hoc analysis (Tukey pairwise comparison) was performed.

**Results**

A total of 34 dogs were included in the study. A total of 24 Boxers were included, which is comprised of 10 dogs in the affected (A) group, 10 dogs in the unaffected (U) group, and 4 in the heart failure (HF) group. Ten normal, non-Boxer (N) dogs were also included. Of the Boxers, the mean age was 6.0 years, with 10 males (42%) and 14 females (58%). Summary data for each individual group is presented in Table 4.1, including the mean values for each parameter in each group of dogs. Results of One-way ANOVA analysis identified differences between the HF group and all other groups (N, A, and U). No differences between affected (A) and unaffected (U) Boxers were identified in any parameter. Variable differences were identified between non-CHF Boxers (A and U) and normal, non-Boxer (N) dogs. A summary of analysis of variance findings is presented in Tables 4.2-4.5. Graphical representations of the
comparisons of heart rate variability parameters (mean RR, SDNN, ASDNN, SDANN) and identification of significant differences between groups are presented in Figures 4.2-4.5.
Figure 4.1  Position of electrodes for Holter placement as seen from both the left and right sides. There are 7 electrodes; 3 pair to create 3 bipolar leads (or channels), and 1 to serve as ground. Channel 1 consists of the red electrode as positive (+) and the white electrode as negative (−), approximating lead II. Channel 2 consists of the brown electrode as positive (+) and the black electrode as negative (−), approximating lead III. Channel 3 consists of the orange electrode as positive (+) and the blue electrode as negative (−), approximating lead I. The green electrode serves as ground.
<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>%Female</th>
<th>VPC#</th>
<th>Mean RR (msec)</th>
<th>SDNN (msec)</th>
<th>ASDNN (msec)</th>
<th>SDANN (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=10)</td>
<td>4</td>
<td>30%</td>
<td>0.6</td>
<td>913.8</td>
<td>411.6</td>
<td>335.1</td>
<td>243.5</td>
</tr>
<tr>
<td>Unaffected (n=10)</td>
<td>3.6</td>
<td>60%</td>
<td>0.7</td>
<td>734.1</td>
<td>292.8</td>
<td>280</td>
<td>171.9</td>
</tr>
<tr>
<td>Affected (n=10)</td>
<td>8.2</td>
<td>60%</td>
<td>4188.7</td>
<td>734.9</td>
<td>327.9</td>
<td>283</td>
<td>175.5</td>
</tr>
<tr>
<td>Heart failure (n=4)</td>
<td>5.8</td>
<td>50%</td>
<td>2385.25</td>
<td>530</td>
<td>151.8</td>
<td>121.8</td>
<td>81.25</td>
</tr>
</tbody>
</table>

Table 4.1. Summary data for age, gender, total number of ventricular premature complexes (VPC#), and heart rate variability parameters including the mean R-R interval (mean RR) and standard deviation of RR interval durations over the entire 24 hour recording period; the mean value for the standard deviation of individual 5 min periods within the 24 hour recording (ASDNN) and the standard deviation of the mean values of individual 5 min periods within the 24 hour recording (SDANN) are also presented.
Figure and Table 4.2. Comparison of mean RR values between groups of dogs. Statistical significance is represented in tabular form as well as by letter designation; groups with similar letters were not found to be significantly different by one-way ANOVA. N=normal non-Boxer dogs, U=unaffected Boxers, A=affected Boxers, HF=Boxers with heart failure.
Figure and Table 4.3. Comparison of mean SDNN values between groups of dogs. Statistical significance is represented in tabular form as well as by letter designation; groups with similar letters were not found to be significantly different by one-way ANOVA. N=normal non-Boxer dogs, U=unaffected Boxers, A=affected Boxers, HF=Boxers with heart failure.
Figure and Table 4.4. Comparison of mean ASDNN values between groups of dogs. Statistical
significance is represented in tabular form as well as by letter designation; groups with similar letters
were not found to be significantly different by one-way ANOVA. N=normal non-Boxer dogs,
U=unaffected Boxers, A=affected Boxers, HF=Boxers with heart failure.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>U</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U</td>
<td>NS</td>
<td>-</td>
<td>0.002</td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>NS</td>
<td>0.003</td>
</tr>
</tbody>
</table>
Figure and Table 4.5. Comparison of mean SDANN values between groups of dogs. Statistical significance is represented in tabular form as well as by letter designation; groups with similar letters were not found to be significantly different by one-way ANOVA. N=normal non-Boxer dogs, U=unaffected Boxers, A=affected Boxers, HF=Boxers with heart failure.
Discussion

This study evaluated the analysis of heart rate variability (HRV) in Boxer dogs with ventricular arrhythmias. The results of this study identified a reduction in heart rate variability in a subpopulation of Boxers with congestive heart failure (CHF). However, affected Boxer dogs with greater than 1000 ventricular premature complexes (VPCs) per 24 hours did not demonstrate any differences in HRV analysis compared to unaffected dogs. In many instances, HRV parameters in normal non-Boxer dogs did not differ from those of non-CHF Boxers, independent of their affected status. In every case, however, Boxers with CHF differed in HRV analysis from all other dog groups, including affected dogs, unaffected dogs, and normal non-Boxer dogs.

The results of this study suggest that HRV analysis in Boxers is similar to studies performed in people, particularly in patients with CHF. In people, patients with CHF have elevated sympathetic tone, causing a reduction in HRV.\textsuperscript{15-22} In fact, the reduction of HRV in patients with CHF is predictive for mortality, especially with dilated cardiomyopathy.\textsuperscript{6,8,23} The assessment of mortality in Boxers with CHF was not performed, partly due to the number of dogs included (n=4), but also because Boxers with CHF and systolic dysfunction are known to have a poor prognosis.\textsuperscript{14,24} The major value of including the subset of Boxers with heart failure is to validate the technique used to assess HRV; the significant reduction of HRV in this group of Boxers was expected, and serves as a marker for increased sympathetic tone.

More intriguing, however, is the assessment of HRV analysis in patients with ventricular arrhythmias in the absence of myocardial failure. The majority of Boxers with ventricular arrhythmias have preserved systolic function, and the nature of the
arrhythmia is not well understood. The formation of ventricular arrhythmias is complex, and is multifactorial in nature. Arrhythmias develop as a result of an arrhythmic substrate that can be modified by several factors. The existence of an arrhythmia may not occur despite the presence of a substrate if the necessary triggers are not present. An important trigger for many arrhythmias is autonomic modulation, particularly sympathetic tone. In Boxers with arrhythmic disease, many reports of syncope or sudden death occur in association with increased activity or excitement, suggestive of an increased catecholamine surge. The utility of HRV analysis in people with ventricular arrhythmias has been shown. The finding in this study that Boxers with frequent arrhythmias do not have reduced HRV may simply reflect a lack of persistently increased sympathetic tone, despite the potential importance sympathetic effects on arrhythmia development. It also appears that in some cases Boxers may have different autonomic patterns relative to other breeds, as evidenced by the variable differences between affected and unaffected Boxers compared to non-Boxer dogs. These findings are supported by previous evaluations of HRV analysis in dogs, where cardiomyopathic Dobermans with mild or moderate ventricular dysfunction did not demonstrate reduced HRV relative to clinically normal dogs. Severe myocardial dysfunction may be required to cause substantial elevations in sympathetic tone in order to overcome the profound normal sinus arrhythmia found in dogs.

The small number of dogs included in the study is a potential explanation of why no differences were identified between affected and unaffected dogs. However, in many instances, the values for HRV parameters were nearly identical in these groups, and the magnitude of differences between both these groups and the normal non-Boxer
dogs and heart failure dogs was at times significant. The normal non-Boxer group was always greater (even if not significantly so) than both the affected and unaffected group, and the heart failure group was always less. Furthermore, the trend between the affected and unaffected group was such that the unaffected group had lower values compared to the affected group; a finding opposite of what would be expected. The low number of heart failure dogs in the study did not prevent identification of significant differences between this group and the other dogs.

The age of the affected group of Boxers was significantly greater than the unaffected group of dogs. It is possible that the inability to identify a difference between the two groups of dogs was because of the effect of age. However, it has been shown that as Boxers get older the number of VPCs increases; therefore, this relationship is expected. Furthermore, the influence of age on HRV analysis in dogs is unknown. Thus, the importance of the age difference between the affected and unaffected dogs is not clear.

In conclusion, the analysis of HRV in Boxer dogs with ventricular arrhythmias is most reduced in those dogs with concomitant congestive heart failure. It is in this group of dogs that sympathetic tone is expected to be increased, particularly in a persistent fashion. The inability to identify a difference between Boxers with and without severe arrhythmias may be due to a lack of persistently elevated sympathetic innervation associated with the presence of arrhythmias, or may reflect the insensitivity of HRV analysis in this group of dogs. It is likely that sympathetic modulation is important in the development or manifestation of ventricular arrhythmias, but may exist in a more phasic or intermittent fashion. Development of other techniques for the
purpose of assessing sympathetic innervation to the heart may be necessary in the evaluation of Boxer dogs with ventricular arrhythmias due to arrhythmogenic cardiomyopathy.
Footnotes

a Cardiocorder cassette recorder, DelMar, Irvine CA

b Accuplus Holter analysis system, DelMar, Irvine CA

c Biomedical systems, St. Louis MO

d Spier AW, Meurs KM, Lehmkuhl LB, Miller MW. Evaluation of ambulatory ECG monitoring in asymptomatic boxer dogs. 17th Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine, June 1999. (abstract)
Reference List


SUMMARY AND CONCLUSIONS

The goal of this project was to examine the clinical utility of non-invasive diagnostic tests in the evaluation of Boxers with ventricular arrhythmias. The impetus behind such an endeavor arose from the current inability to adequately assess the prognosis of affected individuals. Dogs presenting with systolic dysfunction and congestive heart failure represent a minority of patients with the disease, and have a natural history that is more straightforward. These dogs are more likely to suffer a cardiac death, either as a result of sudden death or due to complications associated with congestive heart failure. The more typical manifestation of the disease involves the presence of arrhythmias that may or may not be associated with symptoms of syncope. In this scenario, the course of disease can be profoundly variable, with some dogs suffering from sudden death as a result of their ventricular arrhythmia, to dogs that live a normal lifespan only to eventually succumb to non-cardiac related diseases. Holter monitor evaluation may be valuable in documenting the presence and severity of disease, but is often unable to allow the clinician to evaluate prognosis.

In human medicine, such evaluations are accomplished by use of electrophysiologic testing. While these procedures are helpful in identifying individuals at risk for complications or adverse events, they are inherently invasive, expensive, and not without risk. In veterinary medicine, these procedures can be accomplished, but the
need for anesthesia, expense, and invasive nature has precluded them from widespread use. As a result, identification of less expensive noninvasive diagnostic tests that do not require anesthesia would improve the management of disease. Such tests currently employed in human medicine include Holter monitoring, signal-averaged ECG, assessment of QT dispersion, and analysis of heart rate variability.

Holter monitoring is frequently used to diagnose ventricular arrhythmias in Boxers, and also serves to monitor disease progression and response to therapy. To accurately assess either disease progression or antiarrhythmic effect, the inherent or spontaneous variability of arrhythmia frequency should be known. Parameters exist in human medicine to guide such evaluations, but the degree of variability in dogs has not been reported. The evaluation of spontaneous variability of ventricular arrhythmias in this study resulted in very similar findings to that in people. When frequent arrhythmias are present, less than an 80% change in the number of VPCs may be within the physiologic range of spontaneous variability. Dogs with less frequent arrhythmias may have even more variability in the number of VPCs. However, the grade or complexity of the arrhythmia appears to be less variable than the absolute number. Therefore, when assessing response to therapy or monitoring for disease progression, a change of more than 80% should be identified before documenting a drug response or true disease progression.

The presence of ventricular arrhythmias depends on the existence of a substrate for arrhythmia formation. Altered impulse conduction can create abnormal electrical pathways, which may manifest as reentrant loops or circuits. Reentry is a common mechanism of arrhythmia formation, and identification of abnormal conduction often
heralds the existence of ventricular arrhythmias. Noninvasive evaluation of abnormal conduction can be achieved by the use of signal-averaged electrocardiography. SAECG facilitates the identification of late potentials, which correlate to areas of abnormal tissue depolarization. Documentation of late potentials is associated with increased risk of mortality, and is useful in assessing prognosis and identifying at-risk individuals. The evaluation of SAECG in Boxers proved to be useful in identifying more severely affected individuals that were likely to suffer a cardiac death. However, many of the dogs with late potentials were dogs with poor cardiac function in addition to ventricular arrhythmias. Additional studies that include more dogs will be necessary to identify its utility in dogs with arrhythmias in the absence of myocardial failure. Despite its potential limitations, this technique may prove to be valuable in the evaluation of Boxers with ventricular arrhythmias.

In addition to abnormal conduction, altered repolarization can also contribute to reentry and facilitate arrhythmia formation. Repolarization of cardiac muscle may be directly measured during an electrophysiologic study, but noninvasive assessment of refractoriness can be performed from a surface ECG. Heterogeneity of repolarization manifests on a surface ECG as a difference in the duration of QT duration between leads for any given beat. This so-called QT dispersion correlates with a risk of mortality in people, but did not appear to be as useful in Boxers with ventricular arrhythmias. No correlation between QT parameters and disease severity was identified, and most dogs did not exhibit any dispersion at all (QT dispersion=0). Dogs that did exhibit some degree of dispersion were not dogs with severe arrhythmias, and
the magnitude of dispersion was modest. Therefore, evaluation of QT dispersion does not appear to be a useful diagnostic test in Boxers with ventricular arrhythmias.

The arrhythmia substrate is only partially responsible for the manifestation of arrhythmias. Other factors, including autonomic balance, have important modulating effects on arrhythmia formation. Elevated sympathetic tone exacerbates the tendency for arrhythmia development while parasympathetic tone tends to have a protective effect. One way to assess the degree of autonomic modulation of the heart is to analyze the variation of heart rate. As sympathetic tone increases, the variability in heart rate decreases. Reduced heart rate variability has been used to identify cardiac patients at risk for adverse events, especially patients with dilated cardiomyopathy, coronary artery disease, and ventricular arrhythmias. Analysis of heart rate variability in Boxers revealed that the only reduction in HRV was identified in those patients with congestive heart failure. These patients were expected to have increased levels of sympathetic tone, and served to validate the technique. In contrast, there did not appear to be any value of HRV analysis in Boxers with ventricular arrhythmias and preserved cardiac function. Analysis of HRV in these dogs may not be sensitive enough to identify alterations in autonomic balance, or these dogs may simply not have increased sympathetic tone. Other diagnostics may be necessary to detect changes in autonomic tone in these dogs.

In conclusion, it appears that the use of noninvasive testing to evaluate Boxers with ventricular arrhythmias is limited. Those tests that did identify differences between groups of dogs tended to reflect changes in only the most severely affected individuals. Those dogs that are asymptomatic are not as likely to have abnormal tests,
and continue to represent a challenge in disease management. The use of more invasive
diagnostic testing may be required to fully evaluate these patients and enable the
veterinary clinician to adequately identify patients at risk of developing significant
complications of disease.
BIBLIOGRAPHY


41. Cheng TO. Decreased Heart Rate Variability as a Predictor for sudden Death was known in China in the third Century A.D. *Eur Heart J.* 2000;21:2081-2082.


120. Lander P, Beribari J. Use of high-pass filtering to detect late potentials in the signal-averaged ECG. *J Electrocardiol* 1989;22 (Suppl.):7-12.


125

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


127


