COMPARISON OF AVERAGE HEART RATES DETERMINED BY SURFACE ECG AND 24-HOUR AMBULATORY ECG (HOLTER) IN DOGS WITH SPONTANEOUS ATRIAL FIBRILLATION

A Thesis

by

ADRIANA LUCÍA PEREA LUGO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Biomedical Sciences

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ABSTRACT

Comparison of Average Heart Rates Determined by Surface ECG and 24-Hour Ambulatory ECG (Holter) in Dogs with Spontaneous Atrial Fibrillation.

(December 2010)

Adriana Lucía Perea Lugo, B.S., Autonomous University of Zacatecas

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The purpose of this study was to compare the heart rates of dogs presenting with spontaneous atrial fibrillation (AF) by a surface electrocardiogram (ECG) and a 24 hour ambulatory ECG (Holter recording) in order to determine if there was a difference between these two diagnostic tests. Seven dogs with clinically stable, spontaneous AF were evaluated with a 6 lead surface ECG (MAC 5000, GE® Milwaukee) and a Holter monitor (Monitor device: LifecardCE Delmar Reynolds Medical, Holter analysis:Aria Holter software). Statistical analyses, including t-tests and linear regression models, were performed using Stata® data-analysis and statistical software.

When heart rates (bpm) determined by both diagnostic testing methods were compared individually and among all of the dogs, no statistically significant differences were found. Complete data for analysis were available in 4 of the 7 dogs. This study demonstrates that despite the potential superiority of Holter monitoring relative to the surface ECG for the diagnosis of cardiac arrhythmias, average heart rates were not statistically different in these 4 dogs with controlled AF. Therefore, the average HR determined by surface ECG in the hospital may be as reliable as the average HR determined by Holter monitoring in dogs with well controlled spontaneous AF.

DEDICATION

To my family, teachers, colleagues, friends, and all the people who always supported me during my studies at A&M.

To my students.

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Finally, I would like to thank my entire family for the impeccable example, unconditional support, and encouragement they have always given me.

NOMENCLATURE

AF	Atrial fibrillation
BE	Bicavitary effusion
bpm	Beats per minute
CHF	Congestive heart failure
COPD	Chronic obstructive pulmonary disease
DCM	Dilated cardiomyopathy
DVD	Degenerative atrio-ventricular valve disease
ECG	Electrocardiogram
Holter	Ambulatory electrocardiogram
HR	Heart rate
LD	Lung disease
MR	Mitral regurgitation
ODCM	Occult dilated cardiomyopathy
PH	Pulmonary hypertension
SAS	Subvalvular aortic stenosis
TVD	Tricuspid valve dysplasia
VPCs	Ventricular premature complexes

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1. INTRODUCTION

Atrial fibrillation is the most common supraventricular arrhythmia in dogs, representing 12–16% of all canine cardiac arrhythmias (1). Although it is not a common cause of death, it is considered to have one of the highest morbidities of all the arrhythmias (2). Its pathophysiology is highly complex and is commonly associated with a variety of cardiovascular diseases, in particular structural remodeling and ischemic changes (3) or any condition that causes atrial enlargement such as dilated cardiomyopathy, chronic obstructive pulmonary disease, mitral stenosis, mitral regurgitation, and chronic degenerative atrio-ventricular valve disease. It may also occur secondary to diseases of non-cardiogenic origin, for example in hypothyroidism or during anesthesia. In addition, AF may develop in the absence of any discernible heart disease, being termed *lone* or *idiopathic AF*. It more commonly affects large or giant-breed dogs (Irish Wolfhound, Great Dane, Groendal, Mastiff, and Newfoundland) as opposed to small-breed dogs (4). This may occur because the establishment and maintenance of AF is believed to be related to the mass of the atria; therefore, the larger the dog, the greater the predisposition to develop AF (5).

Dogs with AF may exhibit a wide range of clinical signs, depending on the presence and severity of underlying heart disease. They may present with clinical signs consistent with heart failure, such as cough, dyspnea, ascites, weak pulse, exercise intolerance, weakness, lethargy, inappetance and syncope (4). Sometimes AF is an

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This thesis follows the style of *CanadianJournal of Veterinary Research.*

incidental finding without any cardiovascular clinical sings.

The main goal of therapy in canine patients with AF is to optimize heart rate (HR) and if possible convert, restore, and maintain sinus rhythm (6). Heart rate control is typically achieved with antiarrhythmic medications. The rationale for considering rate control as a treatment option is that hemodynamic impairment caused by the rapid rate often associated with AF may be partially or totally reversed with effective heart rate control, depending on the underlying disease (7). However, in veterinary practice evaluation of HR may be problematic because routine procedures for HR determination such as counting arterial pulses, auscultation of the heart, and an in clinic resting ECG may not adequately predict whether the AF ventricular rate response is well controlled with the selected antiarrhythmic therapy when an animal is stressed in the hospital environment.

In healthy adult dogs, the normal HR may vary between 30 and 200 beats per minute (bpm), primarily as a result of integration of fluctuating autonomic influences on the sinoatrial node (sympathetic stimulation increases the HR, whereas parasympathetic stimulation decreases the HR) (8) and due to many additional factors such as breed, age, physical activity, respiration, thermoregulation, and stress or anxiety (9). The HR in dogs with AF is difficult to monitor and evaluate clinically, because it is irregular and usually very fast.

There are many techniques used to evaluate or measure HR. The methods most commonly used in the hospital setting are palpation of arterial pulse, heart auscultation, and resting surface ECG. Each of these methods has specific limitations including stress to the patient caused by the examination and the short period of time the parameter is assessed (for example a 1 to 2 minute ECG). For these reasons, HR can be difficult to accurately assess and therefore can make it challenging to select appropriate therapy and, determine effectiveness of therapy. (10).

When palpation of an arterial pulse is performed for evaluation of HR in a dog no equipment is needed, which makes it a fast and inexpensive diagnostic tool. The potential inaccuracies in HR estimation are related to two factors. First, the HR is typically rapid and the rhythm is irregular in AF, making it difficult to count. Second, pulse deficits are common in dogs with AF due to the rapid HR and rhythm irregularity related to variation in ventricular filling times and pulse strength, which frequently leads to underestimation of true HR. In addition, stress in the hospital environment may cause an increase in sympathetic tone contributing to an elevation in HR.

Auscultation is a readily available, inexpensive, fast, and practical way of assessing HR. It can be performed by anyone trained to use a stethoscope. However, this technique too is susceptible to inaccuracies and therefore not always reproducible, because of external noise, patient motion, loud respiratory noise or panting and evaluator inexperience. Auscultation is particularly difficult when the HR is fast and irregular, which is frequently the case in AF (11). Finally, auscultation is carried out in the hospital and thus subject to the above mentioned limitations with respect to environmental stress.

The resting surface ECG is considered to be the best method for measurement of HR (12). It is a useful and non-invasive technique that provides critical information regarding the electrophysiological function of the heart that cannot be assessed by other conventional methods (13). However, it may also have limited value because it is typically used only to record a brief period of heart rhythm (1 to 2 minutes, which would only represent 0.07-0.14% of all cardiac depolarizations that occur during a 24-hour period). Furthermore, the resting ECG is performed in the hospital requiring that the dog be restrained in right lateral recumbency with electrodes attached to the skin typically by some form of clips (12) which may cause increased stress, potentially increasing the HR, and making it even more difficult to obtain a reliable or representative HR.

Holter recordings (continuous, 24-hour ambulatory ECG monitoring) represent a way of circumventing many of the limitations in determining average resting HR in dogs with AF that were outlined in previous methods (14, 15) Holter monitoring involves placing a noninvasive medical device with the help of a vest and tape on the dog for an extended period, typically 24 hours, while the dog is allowed to return to his or her normal activity at home. These devices are typically used to diagnose or better define the clinical significance of abnormal heart rhythms, but using them to solely estimate the HR has rarely been reported to assess dogs with AF. One recent study by Colland *et al* reported that Holter monitor testing showed significantly lower HR values than in-clinic ECG for dogs with clinically unstable AF (16). The goals of Holter monitoring in dogs with AF is to accurately determine the average daily HR (17), and correlate clinical signs with the presence, severity, and frequency of other cardiac arrhythmias in the dog's home or natural environment during the course of normal activities for an extended duration (9). The expectation is that this will yield more representative and accurate information and result in improved clinical outcome (18).

Even though the clinical utility of Holter monitoring is well established, its clinical application has some practical limitations. Disadvantages include availability, relatively high client cost, the need for special software, delay in analysis, and the necessity of having trained personal to read and manage the data collection (18, 19). Another limitation is that dogs have to be constantly supervised by their owners during the recording period to avoid chewing or detachment of the device.

The data obtained by this method is of critical relevance to the veterinarian, since medical and therapeutic approaches selected for a dog suffering from AF are dependent on these data (2). In 2004, Gelzer *et al* demonstrated that in hospital ECG poorly represented average HR in dogs with AF when compared to a 24 hour Holter recording from a home environment leading this study to conclude that 24hr Holter monitoring is more accurate (20).

2. MATERIALS AND METHODS

2.1 Dog selection

Seven client-owned dogs previously diagnosed with clinically stable AF were selected from the caseload of the cardiology service at the Small Animal Veterinary Teaching Hospital at Texas A&M University from September, 2006 to May, 2007. Enrollment criteria included the diagnosis of AF that was well controlled by medication. Patients were considered well controlled if no changes in cardiac medications were required on the day of evaluation or during the Holter recording. The sex, age, breed, weight and underlying diagnosis were obtained from the medical record of each dog. An owner consent form was signed by the person responsible for each animal.

2.2 Heart rate monitoring

On the day of enrollment, a complete history was taken and a physical examination was performed by an ACVIM board-certified cardiologist or cardiology resident. Next, a six-lead resting surface ECG was performed on each dog (see Appendix A). The ECG was recorded with the dog gently restrained in right lateral recumbency. Alcohol was applied to the areas of electrode attachment, electrode clips were applied to the dog's limbs and then the ECG recording started. The ECG was recorded for 60-180 seconds. Once the recording was complete, the clips were removed and the ECG was evaluated and saved in the patient file.

For the entire ECG recording period, minimum, maximum, and average HR were calculated manually by the author under a specialist's supervision. The method used to calculate minimum ECG HR was determined by selection of the longest R-R interval which was then converted to HR using the formula; 60 sec / R-R interval (sec) . Maximum HR was determined by selection of the shortest R-R interval and converted to HR using the formula listed above. Finally, ECG average HR was calculated by counting the number of R-R intervals in a representative 6 second interval and multiplying by 10 (paper speed was 25 mm/sec).

Immediately after the ECG, a Holter monitor was placed according to the Reynolds Lifecard system Holter hook-up procedure (Appendix B). Dogs were allowed to return home, and owners were encouraged to continue their normal daily routine and to maintain a diary of medication, sleep, exercise, and other activities thereby allowing evaluation and correlation of the variations in HR based on the animal's behavior.

After 24-hours, the owners were asked to remove the monitors from their dogs and return them by overnight courier to the Texas A&M Small Animal Medical Teaching Hospital. When the device was received, the data from the Holter Lifecard was downloaded and analyzed using Del Mar Reynolds software (Impresario ®).

The recording memory of the Holter monitor was digitized and analyzed by personnel trained in the use of the analysis software. This is a prospective analysis software system that shows every ventricular depolarization (QRS) recorded during the time the device was connected to the dog. Because the HR is typically very fast and irregular with AF, the operator must manually confirm the software's identification of each QRS complex. Accurate identification of each QRS complex is necessary for the software program to provide an accurate summary showing the total number of QRS complexes during the recording period of time, the maximum, minimum, and average HR per hour as well as the number of abnormal beats, with the exact times of their occurrence and length or any paroxysms or pauses.

During manual scanning of the recording numerous artifacts were identified. The rapidity and irregularity of the rhythm resulted in many QRS complexes that were not identified by the software. Therefore, the operator had to manually scan the entire recording for accuracy and identify all QRS complexes that were not identified by the software program in order to assure accurate summaries by the software program. When a question or doubt arose, the cardiologist was contacted to review the report provided by the software program.

Summary information was generated by the software program as outlined below.

1. Determination of overall minimum HR. The minimum HR was determined from a 60 second interval. The interval was selected by the software program. The program determined the HR for the initial 60 seconds of the recording and then determined the HR from interval 2 (01 second to 61 seconds) then interval 3 (02 -62 seconds) and so on through the entirety of the recording (rolling 60sec average). The minimum HR was then selected from all available 60 second intervals.

2. Determination of overall maximum HR. The maximum HR was determined from a 60 second interval. The interval was selected by the software program. The program determined the HR for the initial 60 seconds (0-60 seconds) of the recording, then determined the HR from interval 2 (01 second to 61 seconds) and then interval 3

(02 -62 seconds) and so on through the entirety of the recording (rolling 60 sec average). The maximum HR was then selected from all available 60 second intervals.

3. Determination of overall average HR. The average HR was determined from the average of all 60 second intervals. The program determined individual 60 sec interval HR as follows: HR for the initial 60 seconds (0-60 seconds) of the recording, then determined the HR from interval 2 (01 second to 61 seconds) and then interval 3 (02 -62 seconds) and so on through the entirety of the recording.

4. Hourly Holter HR parameters were determined as outlined above for overall Holter HR parameters but instead of evaluating the entire recording the Holter was analyzed in 1 hour intervals.

2.3 Statistical analysis

Numeric variables were described by mean, standard deviation, and range. Holter and ECG average, minimum and maximum HRs were compared using t-tests for each dog who had both Holter and ECG data available. To compare the differences between Holter and ECG average, minimum and maximum HRs, a linear regression model with a random effect for each dog was used. All analyses were performed using Stata® version 10.1 software.

3. RESULTS

3.1 Descriptive statistics

The mean age and body weight of all dogs was 6.78 years (Range 3.0 –13.0) and 48.3 kg (Range 23.0-73.5), respectively. There were five males and two females of the following breeds: Great Dane (3), Rottweiler (2), Poodle (1), and mixed (1) (Table 1).

Table 1. Dog demographics

Dog #	**Gender**	**Age (years)**	**Weight (kg)**	**Breed**	**Underlying diagnosis**	**Current medication***
1	Male	5.0	73.5	Great Dane	ODCM, MR, VPCs	d
2	Male	6.0	47.5	Rottweiler	DCM, CHF, BE	d,r,m,p,a,f
3	Female	6.0	39.3	Rottweiler	SAS, CHF, Hypothyroidism	d,m,p,a,n,l,g,j,q, t,o
4	Female	11.0	28.0	Poodle	DVD, CHF, PH,	k,m,r,p,c,s,h,b,i
5	Male	13.0	23.0	Mixed	DVD, PH	k,m,r,p,c,s,h,b,i
6	Male	3.0	58.90	Great Dane	TVD	p j,d,g
7	Male	3.5	68.18	Great Dane	ODCM, LD	d,a,e
	Mean	6.78	48.34			
	Stand. dev.	3.78	19.48			

* a) Amiodarone, b) Aspirin, c) Atenelol, d) Benazepril, e) Cephalixin, f) Dilacor, g) and h) digoxin i) Doxycycline, j) Diltiazem, k) Enalapril, l) Famotidine, m) Furosemide, n) Levothyroxine, o) Metronidazole, p) Pimobendan, q) Prednisone, r) Spironolactone, s) Sildenafil, t) Sucralfate.

The surface ECGs were read and interpreted in 5 dogs, giving three separate measures of heart rate. The average duration of the surface ECG was 100.8 seconds with a range of 81.0 to 126.0 seconds (Table 2).

Table 2. ECG duration

Dog #	**ECG duration (sec)**
1	108
2	81
3	84
4	No ECG available
5	No ECG available
6	126
7	105
Mean	100.8

The mean minimum HR of all dogs was 85 bpm and the mean maximum HR was 194 bpm. The mean average HR for all dogs was 124 bpm (Table 3).

Table 3. ECG heart rate

Dog #	**ECG minimum**	**ECG average**	**ECG maximum**
1	81	132	214
2	100	121	167
3	69	95	188
4	--	--	--
5	--	--	--
6	48	115	188
7	103	150	214
Mean	80.20	122.6	194.20
Stand.dev.	22.77	20.38	20.00

Holter recording HR was reported as three separate measures:

The mean minimum Holter HR for all hourly readings for all six dogs was 100 bpm (Range 61-155). The mean maximum HR for all hourly readings for all six dogs was148 bpm (Range 90-212), and the mean average HR for all hourly readings for all six dogs was 118 bpm (Range 79-170) (Table 4).

Table 4. Holter heart rate

Dog #	Holter minimum	Holter average	Holter maximum
1	--	--	--
2	102	151	200
3	73	157	212
4	76	155	208
5	69	164	173
6	61	83	189
7	146	156	167
Mean	87.83	144.33	191.5
Stand. dev.	31.68	30.34	18.51

Figures 1 through 4 show the minimum, average, and maximum HR data collected on the four dogs in which both Holter and ECG data were available, by time, in hours. Circular markers observed are Holter HRs. Straight solid lines are mean Holter minimum (green), mean Holter maximum (red) and the mean Holter average (blue).

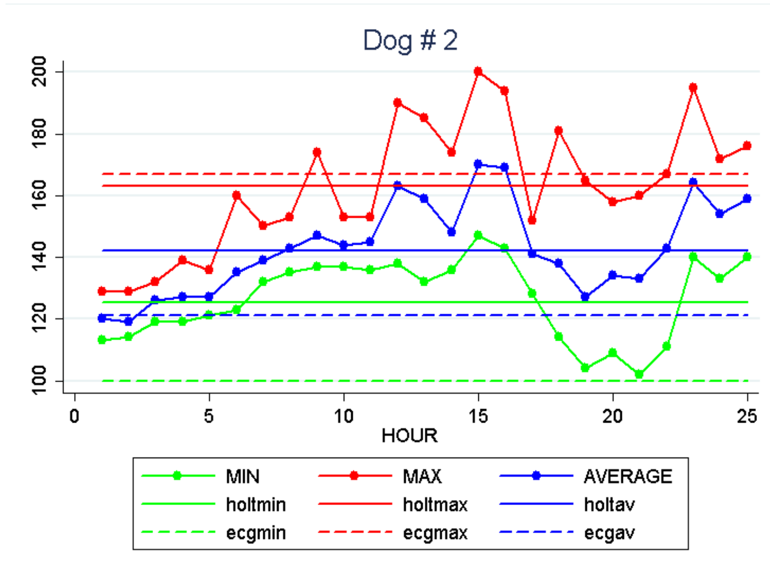

Figure 1. Dog # 2 ECG and hourly HR

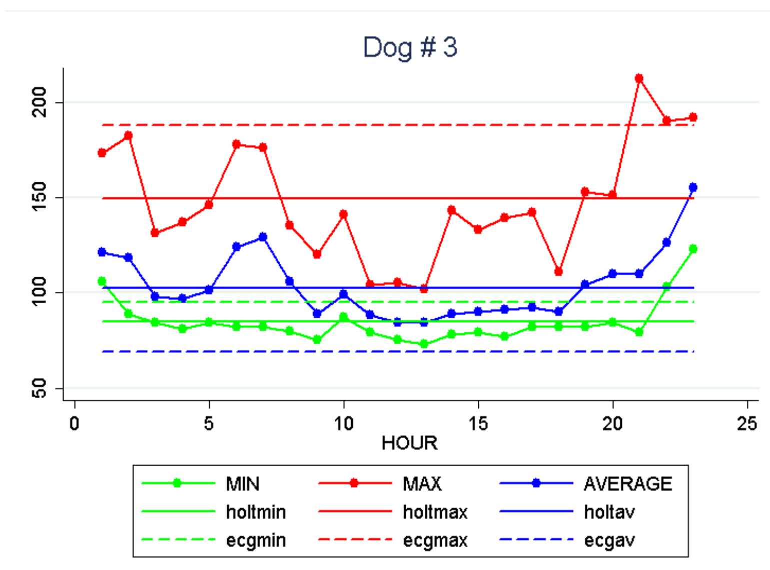

Figure 2. Dog # 3 ECG and hourly HR

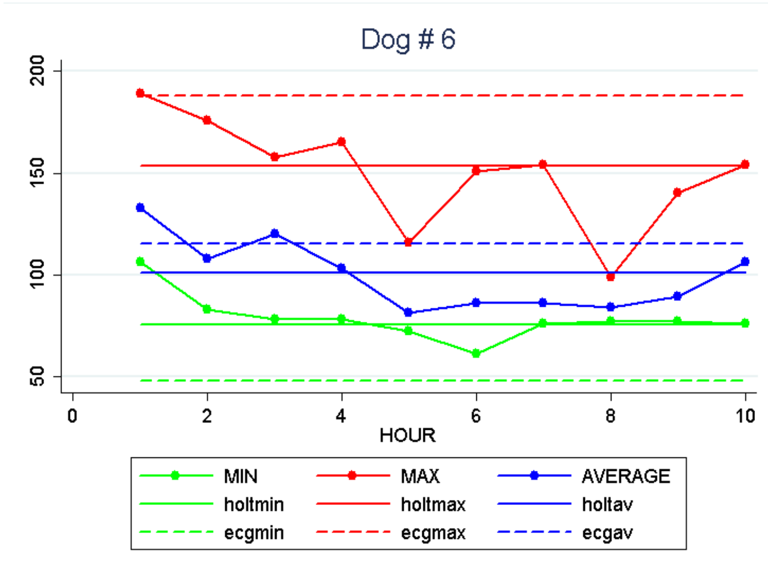

Figure 3. Dog # 6 ECG and hourly HR

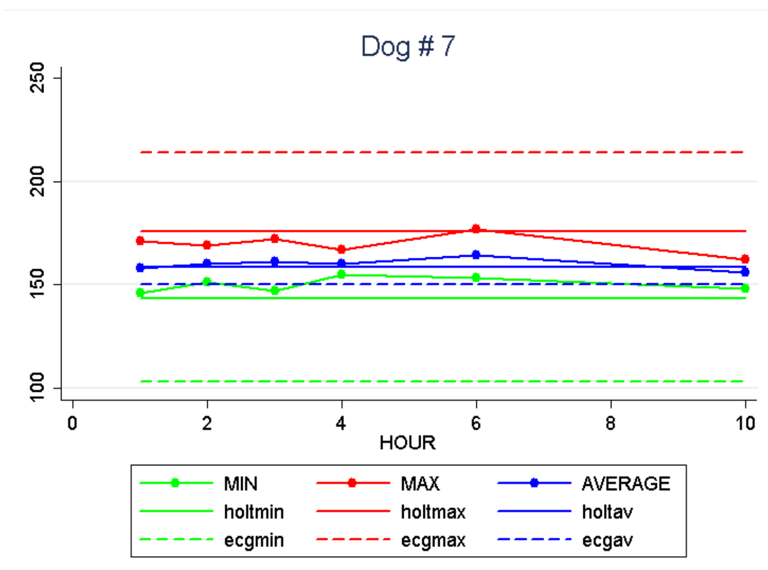

Figure 4. Dog # 7 ECG and hourly HR

3.2 Statistical analysis

The comparison analyses were only carried out on the five dogs that had both Holter and ECG data available. The dogs' HRs were analyzed individually using a one-sample t-test to compare Holter HRs to the ECG HR. Three variables for both ECG and Holter were used, giving a total of nine possible different comparisons per dog (see Table 5).

Table 5. Summary of the p values from t-tests comparing Holter to ECG by dog.

Comparison		Dog #2	Dog #3	Dog #6	Dog #7
N (# Holter obs)		26	24	11	7
Holter	ECG	p value	p value	p value	p value
min	min	0.00	0.00	0.00	0.00
min	avg	0.11	0.00	0.00	0.36
min	max	0.00	0.00	0.00	0.00
avg	min	0.00	0.06	0.27	0.00
avg	avg	0.00	0.00	0.02	0.00
avg	max	0,00	0.00	0.00	0.00
max	min	0.00	0.00	0.00	0.00
max	avg	0.00	0.00	0.00	0.01
max	max	0.36	0.00	0.00	0.00

There are significant differences for at least one dog for each comparison. The only comparisons showing any lack of significance (that is, at least one comparison with a large p-value) are

- Holter minimum compared to ECG average,
- Holter average compared to ECG minimum,
- Holter maximum with ECG maximum.

To compare Holter and ECG HRs for all 4 dogs together, a linear regression model with a random effect for each dog was used, with the response variable being the difference between a Holter HR and an ECG HR (ECG-Holter). The results are listed in Table 6 and estimated differences are displayed graphically in Figure 5, together with 95% confidence intervals.

Table 6. Summary of the p values, sample size N and estimated difference from linear regression comparing Holter to ECG (ECG-Holter).

	Holter minimum			**Holter average**			**Holter maximum**		
	p	N	ECG-H	p	N	ECG-H	p	N	ECG-H
ECG min	0.06	64	-23	0.00	64	-40	0.00	64	-71
ECG avg	0.74	64	4	0.22	64	-13	0.00	64	-45
ECG max	0.00	64	79	0.00	64	63	0.00	64	31

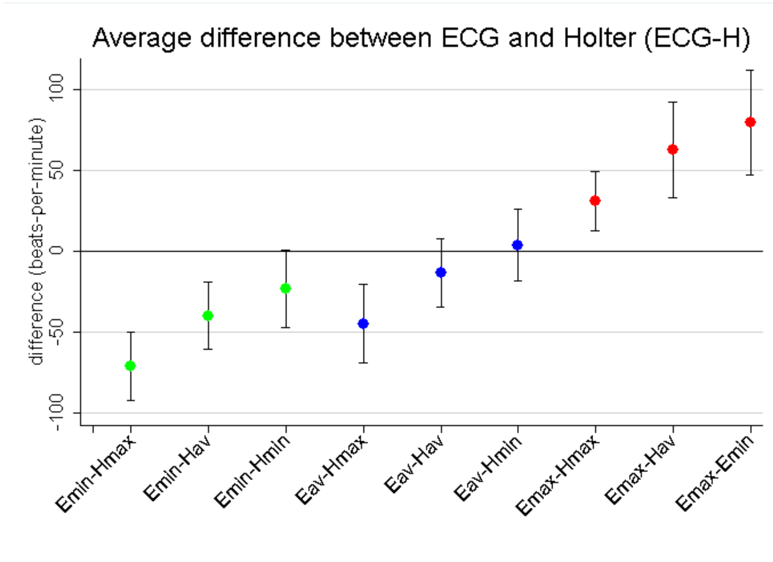

Figure 5. Estimated average difference between Holter and ECG HRs (ECG-Holter), with 95% confidence interval.

This analysis shows that the difference between the Holter average HR and the ECG average HR are not statistically significantly. The difference between Holter minimum and ECG average was not significant at the 5% level, and the estimated average difference between them is only 4 beats-per-minute. The estimated average difference between average ECG and average Holter HR is 13 beats-per-minute.

4. DISCUSSION

Atrial fibrillation is one of the most common arrhythmias that requires treatment in veterinary medicine (21). The treatment goal in dogs with this arrhythmia is typically to optimize HR (22). Many studies have reported the use of Holter recordings and the surface ECG in dogs with cardiac diseases, but at the time this study was initiated there were no prior publications of the comparison of these two methods for evaluating HR in dogs affected with spontaneous AF. This study suggests that there is not a significant difference between the average HR calculated by these two methods in clinically stable spontaneous AF. However, recent abstracts and convention proceedings have reported that average HR determined by Holter is lower than those determined from ECG and is thus more accurate. (16, 20) However, these patient populations were not well described do to the limited nature of abstract publications and may not represent the same population of dogs reported in this study which could explain the apparent discrepancy.

Studies that compared Holter and ECG for evaluation of HR in dogs with sinus rhythm reported that the average HR from the surface ECG consistently exceeded the average Holter heart rate (15). Another previous veterinary study by Gelzer *et al* suggested ECG is not an accurate technique to estimate HR in dogs with AF (23).

Potential limitations of this study include the following:

4.1 Sample population

The dogs for this study were not a homogeneous sample, the only inclusion criterion was a previously diagnosed AF that is stable and not in need of adjustments of cardiac medications on the day of the evaluation or while wearing the Holter. But prior studies in dogs have evaluated the influence of sex, age, heart rate, and body position during ECG, and they showed by statistical analysis that there was not a physiological difference observed in the minimum, median, and maximum cardiac rates (13, 15). Nevertheless, the sample, composed of four animals, is by no means representative of all dogs affected with AF.

4.2 Client and owner

The device requires constant owner attention to avoid detachment of the device from the dog. Even though clients were encouraged to follow normal daily activities at the time of ambulatory ECG recording, many of them reported in the diaries they kept that excessive attention was given to the dog and thus it avoided its normal behavior. Anxiety or stress from having the Holter monitor attached could also affect the HR and lead to overestimation of the HR.

Although the Holter technique does not imply any risk to the animal, some discomfort may be provoked, which would result in the removal of the device, giving at the time of reading many artifacts or loss of data. So as not to cause irritation or alert, owners must be prompted to the fact that when the electrodes are removed a mild erythema will sometimes be present at the site of detachment.

4.3 Holter device and software

Because the software used to read the Lifecard of Holter was designed for cardiac evaluation in humans, interpretation errors are possible when it is used on veterinary patients. For example, in dogs, the average normal HR is higher than in humans, and in dogs with AF it is even higher, therefore missing data by the program has a high probability of occurring. Our goal was to have a 24-hour Holter recording and an ECG for each dog, but the Holter reading was not clear or readable for this period in all cases.

In addition, the limitations of this Holter software made the analysis of each recording labor-intensive since each missed beat had to be manually confirmed by the operator. Some of the dog Holter analysis readings took 3 hours, whereas some took up to a week to review.

It is almost certain that the limitations mentioned above will be overcome with time and fine-tuning of technology for evaluation of canine studies in such a way that this method will be applied with the same frequency and accuracy that has been accomplished in the field of human medicine. In addition, it will be necessary to specify optimal parameters and conditions for evaluation, to decrease the Holter recording time and specify during which dog’s activities. Finally, it will also be very important to determine the situations in which the use of the Holter method is strictly necessary so as to promote the diagnosis of cardiac alterations and improve its use in therapeutic studies.

5. CONCLUSIONS

The results of this study show that there was no significant difference when comparing the average Holter HR versus average ECG heart rate in dogs with clinically stable spontaneous AF in 4 dogs. The importance and clinical relevance of this conclusion is that surface ECG HR recorded at the hospital appears to be as reliable as when a Holter device is applied for hours in dogs with stable spontaneous AF.

Despite the results, the limitations of this study regarding the number of dogs and the software must be noted. It is highly probable that the accuracy of the Holter software analysis will be improved for its use in small animals. And when this occurs, more studies regarding AF or other kinds of disturbances in cardiac electrical activity and its monitoring will be better understood, and more important they will be controlled giving to the dogs affected a better quality of life, as well as for their owners.

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APPENDIX A

ECG monitoring

1. The ECG was recorded with the dog lying on its right side. No sedation or anesthesia was used in the dogs for this study in order to avoid any drug influence on the heart.

2. Conductive gel was applied to the skin of the dog for a better transmission of the electrical activity of the heart to the ECG machine.

3. The electrodes were attached to the chest wall, and after that to the lead cables that were connected to the ECG machine.

4. The activity generated by the heart was recorded for 3 min.

5. Once the recording was completed, the clips were removed.

6. ECG data were evaluated with the help of a cardiology specialist at the Texas A&M Small Animal Hospital.

APPENDIX B

Holter placing and monitoring

1. The hair-coat was shaved over the area where the heartbeat is palpated for a better contact of the skin.

2. At that site, the skin was cleaned and degreased using alcohol and allowed to dry completely.

3. Using an ECG self-adhesive electrode, the red electrode was placed on the left side of the chest and attached over the left apex beat.

4. With the help of a second adhesive electrode placed on the left side of the chest (slightly higher up the rib space and 1–2 rib spaces further forward than electrode 1) the yellow electrode was attached.

5. A third electrode was positioned on the right side of the chest, where the heartbeat was feeling best. The green electrode was attached to this area. (Figure 1)

6. A bandage was used over the electrodes and the leads, once around the chest to ensure there was no interference or movement of these electrodes. Making sure that the dog was not bandaged too tightly or heavily, a specially designed jacket was then used to affix the Holter recorder to the dog.

7. After assuring that the Holter was secure and the dog the most comfortable possible, the recording of the Holter was started.

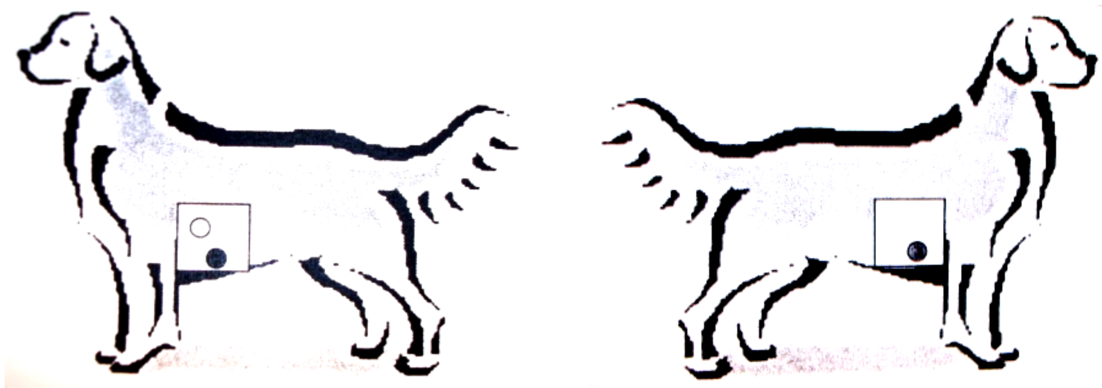

Figure 6. Diagram showing the correct sites of holter electrode application in a dog.

APPENDIX C

ECG equipment

General Electric

GE Marquette

MAC 5000

Milwake, USA

Holter equipment

LifecardCF Delmar Reynolds, USA

Software equipment

Delmar Reynolds software (Impressario ®), USA.

Statistical analysis software

Stata® version 10.1

StataCorp LP,

4905 Lakeway Drive,

College Station Texas 77845

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